

AUG 16 1923

THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

	PAGE
THE EXCRETION OF WATER BY THE KIDNEYS. <i>Edward F. Adolph</i>	419
THE INFLUENCE OF COMBINATIONS OF INORGANIC SALTS AND OF VARIATIONS IN HYDROGEN ION CONCENTRATION ON THE HELIOTROPIC RESPONSE OF ARENICOLA LARVAE. <i>R. S. Little and C. E. Shepard</i>	450
INSULIN AND GLYCOLYSIS. <i>G. S. Eadie, J. J. R. Macleod and E. C. Noble</i>	462
PHYSIOLOGICAL EVIDENCE OF THE EXISTENCE OF A NON-VISUAL AFFERENT MECHANISM IN THE EYE. I. VASOMOTOR RESPONSES TO INTENSE LIGHT. <i>C. I. Reed</i>	477
AFFERENT RELATIONS OF THE SKIN AND VISCERA TO THE PUPIL DILATOR MECHANISM. <i>Joseph Byrne</i>	482
BIOLOGICAL FOOD TESTS. III. CHANGES IN VITAMINS A AND B OF THE GLOBE ARTICHOKE DUE TO VARIOUS CANNING AND DRYING PROCESSES. <i>Agnes Fay Morgan and Helen D. Stephenson</i>	491
STUDIES ON THE PATHOGENESIS OF TETANY. III. EXCITING FACTORS IN EXPERIMENTAL TETANY IN DOGS. <i>Lester R. Dragstedt, Kenneth Phillips and A. C. Sudan</i>	503
A STUDY OF THE SIMULTANEOUS CHANGES IN BLOOD PRESSURE IN THE CAROTID ARTERY AND JUGULAR AND PORTAL VEINS IN ANAPHYLACTIC AND PEPTONE SHOCK IN THE DOG. <i>J. P. Simonds</i>	512
THE EFFECT OF HIGH AND LOW TEMPERATURES ON THE CATALASE CONTENT OF PARA- MECIUM AND SPIROGYRA. <i>W. E. Burge</i>	527
STUDIES ON THE VISCERAL SENSORY NERVOUS SYSTEM. XV. NOTE ON THE INNERVATION OF THE CARDIA IN THE MACACUS MONKEY. <i>A. J. Carlson and S. Litt</i>	534
STUDIES ON RENAL TUBULE FUNCTION. III. OBSERVATIONS ON THE EXCRETION OF SULPHATE, WITH A MODIFIED TECHNIQUE FOR THE DETERMINATION OF INORGANIC SULPHATE IN BLOOD OR PLASMA. <i>H. L. White</i>	537
THE SENSIBILITY OF THE EYE TO DIFFERENCES IN WAVE-LENGTH. <i>Henry Laurens and W. F. Hamilton</i>	547
THE SENSIBILITY OF THE FATIGUED EYE TO DIFFERENCES IN WAVE-LENGTH IN RELATION TO COLOR BLINDNESS. <i>W. F. Hamilton and Henry Laurens</i>	569
TEMPORAL VARIATION IN THE FUNCTION OF THE GYRUS PRECENTRALIS IN PRIMATES. <i>K. S. Lashley</i>	585
CONDITIONED REFLEXES AND PATHWAYS IN THE SPINAL CORD. <i>Jessie M. Lang and J. M. D. Olmsted</i>	603
INFLUENCE OF TEMPERATURE CHANGES ON THE SECRETION OF EPINEPHRIN. <i>F. A. Hart- man and W. B. Hartman</i>	612
THE PRODUCTION OF EPINEPHRIN BY THE ADRENAL CORTEX. <i>F. A. Hartman and W. E. Hartman</i>	623
INDEX.....	635

VOL. LXV—No. 3

Issued August 1, 1923

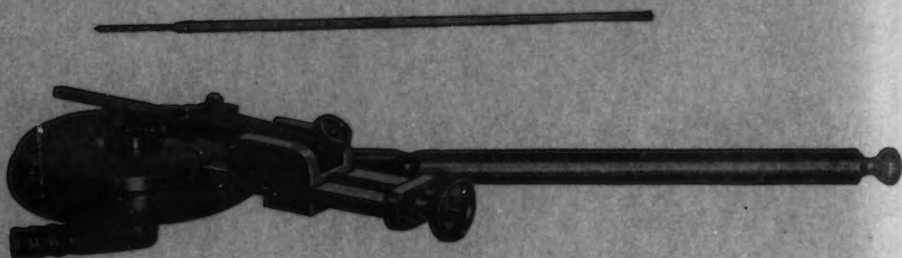
BALTIMORE, U. S. A.

1923

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VOL. 65

AUGUST 1, 1923

No. 3

THE EXCRETION OF WATER BY THE KIDNEYS

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From the Zoological Laboratory, University of Pittsburgh

Received for publication May 1, 1923

I. INTRODUCTION. The nature of those activities of the kidneys which are illustrated by the excretion of water, has not been overlooked merely because of the prevailing idea that water is in the urine passively, but because, as Ambard (1, p. 11) points out, no method has been found for the study of its excretion.

A study of human urinary concentrations has brought to light certain quantitative relationships regarding the kidneys' output of dissolved substances. Using these relationships as a basis, it is now possible to compare the water excretion with that of the dissolved substances, the excretion of which is already partially understood. From these results can be deduced principles which serve, at least, to account for several fundamental properties of the kidneys' activities, particularly for those concerned in diuresis.

1. *Acknowledgments.* It is a pleasure to acknowledge my indebtedness to Dr. J. S. Haldane in Oxford and to the U. S. Bureau of Mines in Pittsburgh for generously placing at my disposal the facilities of their laboratories. For helpful discussion of several points in the investigation I owe gratitude to Doctor Haldane, Mr. J. B. S. Haldane and Dr. G. Stegeman. The later experiments could hardly have been performed except for the kindly interest of Dr. R. R. Sayers and Mr. W. P. Yant.

2. *Methods of experimenting.* Experiments were performed at various times over a period of two years upon a single human subject (E. F. A.), weighing 70 kilograms, and in excellent health. Conditions of bodily activity were made as uniform as possible during the 8 or 10 hours of each experiment, and indeed continuously through successive 24-

hour periods. No meals were taken during each experiment and, after the ingestion of some solution, complete voluntary urine samples were collected at hourly intervals, or occasionally more frequently. On representative control days the rates and concentrations of urinary excretion were measured with the same accuracy, and from these observations the normal factors and variations were discovered. Experiments 1 to 27 were done in Oxford, and experiments 28 to 51 in Pittsburgh.

Urine volumes were measured in graduated cylinders in which the samples had been collected. Chlorides were titrated by Harvey's (2) modification of the Volhard method, urea by the clinical method of Marshall (3), and ammonia by the formol titration of Malfatti (38). Bicarbonate was measured gasometrically in the small blood gas analyzer of Haldane (4). The hydrogen ion concentration of urine was roughly observed by its turbidity, by its reaction to litmus, or occasionally by colorimetric comparison with the phosphate standards of Sørensen (5). Freezing point determinations were made with a Beckmann thermometer graduated to 0.01°C.

II. MAXIMAL URINARY CONCENTRATIONS. The quantities of dissolved substances in urine have, naturally, been measured relative to the quantity of water excreted. If, on the contrary, in studying water output, water is to be referred to any other substance as a measure of concentration, we must first know some of the laws governing the excretion of that other substance. Ambard (1) has discovered two such laws for dissolved substances, namely, that for every secretory organ there is a maximal concentration beyond which its product cannot be elaborated; and, that a constant relationship exists between the concentration of each substance in the blood and the rate of its excretion at a given moment. These two principles furnish the necessary information concerning, for example, chloride or urea, so that to them the water concentration may justly be referred.

1. The maxima for chlorides and for urea. We may first examine the manner of excretion of dissolved substances. It is of supreme importance to know that there is a limit which the kidneys obey in concentrating dissolved substances during excretion, and a number of experiments were performed to discover the nature and range of validity of this maximum. These experiments have been already briefly reported (6). Substances such as sodium chloride and urea were ingested in large amounts with very small quantities of water, and the concentrations of each, in the urinary diuresis which followed, were determined.

Typical experiments are shown in figures 1, 2 and 3. After the ingestion of sodium chloride, the results of which are shown in figure 1, the constancy of the chloride concentration in urine during a period in which the output of water and of chlorides varied greatly, indicates the pre-eminent importance of the concentration factor. In similar experiments and in others in which urea was ingested (fig. 2), it is likewise remarkable that the chloride concentration and the urea concentration,

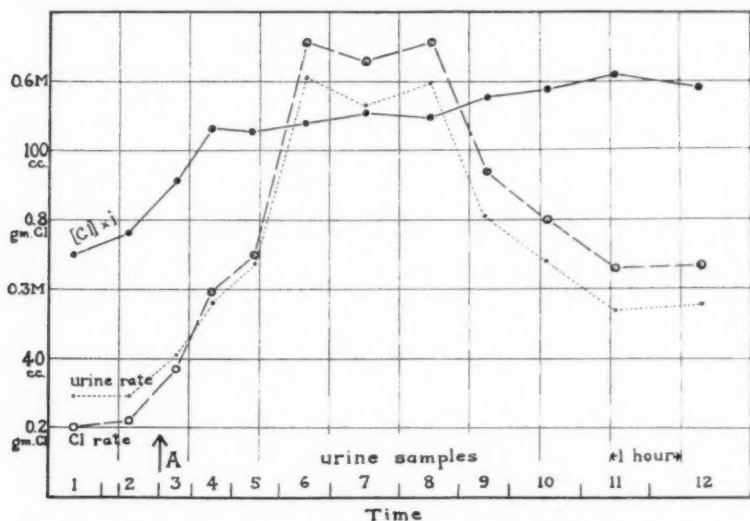


Fig. 1. Experiment 28. Graphic representation of the diuresis and of chloride excretion after ingesting 28 grams of NaCl at A. Chloride concentration in all experiments has been calculated as NaCl, and has been corrected for ionization, i , by graphically interpolating the standard values of the isotonic coefficient (30). Data are represented as points in the middle of the time interval during which the urine sample accumulated in the bladder. Maximal concentration attained in samples 3 to 12, highest in sample 11. Urine rate in cubic centimeters per hour.

respectively, attained constancy before the increase of water output denoting diuresis had reached its maximum. Bicarbonate ingestion (fig. 3) gave a plateau in the urinary concentration of bicarbonates, which can hardly be accounted for on any other hypothesis (7, pp. 23 and 129) than that there is a limiting concentration for the kidneys' excretion.

2. *Factors influencing the maxima.* Ambard states that the only factor which may influence the maximal concentrations is a change in the quality of the renal tissue, such as occurs in nephritis (1, p. 49). I have found at least two other factors. First, in prolonged thirst chlorides were excreted at concentrations 20 to 30 per cent above the normal maximum (fig. 5). This one expects, since the urinary concentration is obviously relative to blood concentration, and hemoglobin determinations showed an increase of 8 to 12 per cent in the hemoglobin content of the blood during the thirst periods.

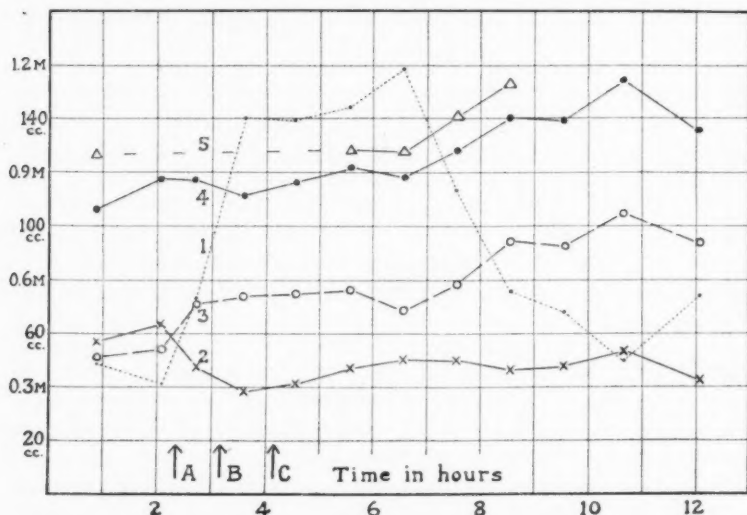


Fig. 2. Experiment 30. Ingesta: 30 grams urea at A, 15 grams urea at B, 15 grams NaCl at C. Curves of urine data: 1. Rate of urine excretion in cubic centimeters per hour. 2. Chloride concentration corrected for ionization. 3. Urea concentration. 4. Sum of 2 and 3. 5. Depression of freezing point (uncorrected). Total maximal concentration attained in sample 11.

Second, two substances calling for excretion in large amounts at one time lower the maximum for each other. Such cases were investigated by Ambard (10), and have been found by Chaussin (8) and by J. B. S. Haldane (9). Experiments with the same substances as Haldane used, bicarbonate plus chloride (fig. 3), proved to yield the same total maximum either separately or simultaneously. Bicarbonate excretion

following exposure to high temperatures (experiments 50 and 51) gave this total, too. This interference regularly occurs in herbivorous animals (37). In the case of urea and chloride, studied by Ambard (10) and by Chaussin (8), the interference is less obvious. Nevertheless several experiments which I have performed demonstrate that there is interference such as Chaussin concluded to exist. Haldane, however, does not believe that either urea or phosphates interfere with the concentration of chlorides during their excretion. In figures 2 and 4 it will be seen that under conditions where both chlorides and urea were excreted

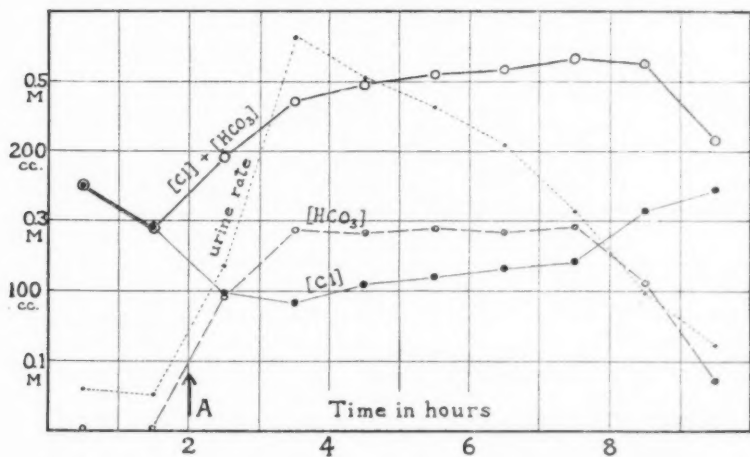


Fig. 3. Experiment 22. Maximal urinary concentrations of chloride plus bicarbonate after ingesting 20 grams NaCl and 28.5 grams NaHCO₃ at A. Urine rate in cubic centimeters per hour, all concentrations corrected for ionization.

rapidly, the highest concentrations of one accompanied lower concentrations than the maximum characteristic of the other. The interference, however, is not quantitatively like that of chlorides and bicarbonates, and for a given pair of kidneys the total maximum is not an unvarying quantity. Ambard's ability to recognize its existence, amid its many variations, is characteristic of the recognition of our most important physiological principles.

I have found three chief types of variation in the maximum, in addition to the two mentioned above: *a*, a daily variation, which is surprisingly small (5 per cent), perhaps concerned with the activities and the

water level of the entire body; *b*, a variation correlated with the work of the kidneys immediately preceding, such as is involved in prolonged diuresis; *c*, a variation with long stretches of time, as from year to year. All of these variations are indicated in figure 5, in which are plotted the highest chloride concentrations attained for each experiment in which chlorides were ingested.

Undoubtedly other factors enter in as well; the chief point is that the maximum when actually tested under the most varied conditions was

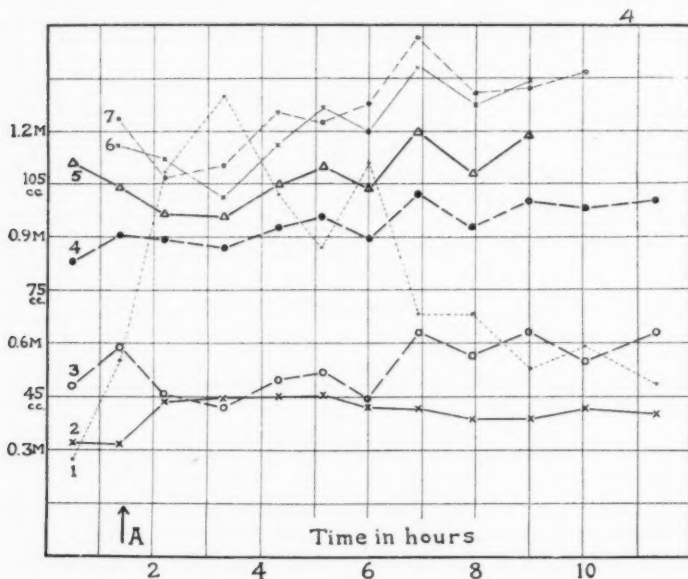


Fig. 4. Experiment 32. Urinary concentrations after ingesting 15 grams NaCl and 25 grams urea at A. 1. Urine rate in cubic centimeters per hour. 2. Chloride concentration, corrected for ionization. 3. Urea concentration. 4. Sum of 2 and 3. 5. Depression of freezing point (uncorrected). 6. Specific gravity (sp. gr. - 1). 7. Residue dried at 100°C. Curves 6 and 7 in arbitrary units. Note especially the parallelism of curves 4, 5 and 6.

always within 30 per cent of its highest extreme value. Under identical conditions, seven experiments with chloride ingestion gave an average concentration of 0.298 M chlorides (or 0.526 M when corrected as NaCl for electrolytic dissociation). The extremes varied by only 7 per

cent in spite of the fact that the doses varied from 0.17 to 0.51 gram molecules of chloride. The variation in the maximum caused by the amount of chloride ingested is seen in figure 5 to be relatively slight, and was due to the length of time during which the excessive excretion continued.

A similar series of experiments was performed in which urea was ingested, with parallel results.

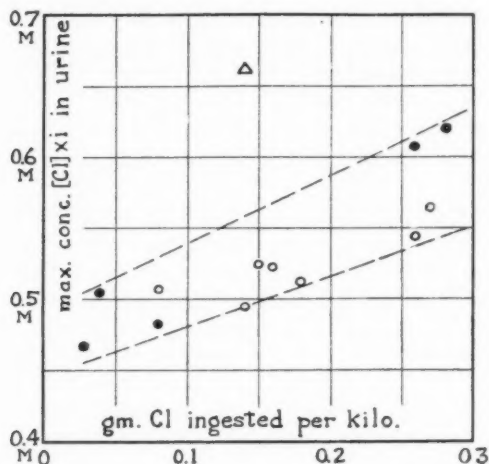


Fig. 5. Highest concentrations attained after ingesting various amounts of sodium chloride. Five experiments in Pittsburgh, 7 experiments in Oxford, 1 experiment on fourth day of thirst in Oxford.

3. *Individual and collective maxima.* Is it possible to account for the discrepancy between the supposed maximum for chlorides and that for urea? On separate days under identical conditions osmotically equivalent quantities of chloride and of urea were ingested. The maximum for urea in the urine turned out to be 0.678 M, and that for chlorides 0.522 M (corrected for ionization as NaCl (30)). The difference, 0.156 M, is approximately the osmotic pressure due to the threshold concentration of sodium chloride retained in the blood, and against this, of course, the kidneys have to work. During thirst both rose proportionally, the urea maximum being 0.782 M and the chloride 0.662 M. In other cases the figures (shown in table 1) are less satisfactory from the standpoint of this suggestion.

TABLE 1
Maximal urinary concentrations after ingestion of various salts. Does not include eleven of the chloride experiments shown in figure 5

EX- PERI- MENT NUM- BER	SUBSTANCE INGESTED	AMOUNT INGESTED, GRAMS PER KILO	MAXIMAL SALT CONCENTRATIONS, M	MAXIMAL SALT CONCENTRATIONS CORRECTED FOR IONIZATION M	MAXIMAL SALT CON- CENTRA- TIONS $[\text{Cl}] \times 1 +$ $[\text{HCO}_3] \times 1 +$ $[\text{urea}]$	REMARKS
38	Urea	0.03 N	0.603		0.948	
16	Urea	0.20 N	0.678		0.839	
18	Urea	0.20 N	0.634		0.798	During water privation
19	Urea	0.30 N	0.782		0.928	Privation continued
29	Urea	0.21 N	0.828		1.061	
12	NaCl	0.14 Cl	0.369	0.644	0.837	During water privation
31	NaCl	0.28 Cl	0.353	0.620	0.854	$\Delta = 0.34 \text{ M}$
33	NaHCO ₃	0.45 HCO ₃	0.374	0.617	0.782	$\Delta = 0.87 \text{ M}$
22	NaCl + NaHCO ₃	0.17 Cl + 0.29 HCO ₃	0.133 + 0.174	0.243 + 0.297	(0.540)	Urea not determined
23	NaCl + urea	0.12 Cl + 0.19 N	0.220 + 0.405	0.396 + 0.405	0.801	
30	NaCl + urea	0.14 Cl + 0.32 N	0.219 + 0.765	0.394 + 0.765	1.159	
32	NaCl + urea	0.14 Cl + 0.18 N	0.230 + 0.630	0.411 + 0.630	1.041	$\Delta = 1.19 \text{ M}$

The remarkable thing about the kidneys' activities is that they deal with each substance as if they were independently stimulated to remove the excess of each from the blood. Nevertheless the same maximal concentration value holds true for each of the chemical individuals which are being excreted. From the data of other authors (1), (11) it is probable that the same secretory constant, also, is valid for all substances.

The real question is: Do all substances serve to limit the total concentration of dissolved substances in urine by virtue of their particulate concentration? This was studied by measuring the depression of the freezing point in concentrated urines. Whereas no maximal value was found, the limits were always approximately the same. In four diuresis experiments the largest depressions found in each were equivalent to: 1.15 M, 1.05 M, 1.19 M and 0.94 M.

The total concentrations can be measured less exactly by simply determining the specific gravity of the urine, or the residue after drying at 100°C., both of which were found to correlate well with the freezing point values. The correlations are shown in figure 4. The sum of the urea and chloride concentrations amounts to 90 per cent of the total concentration of substances in the urine, during a diuresis due to either one of them.

The failure to recognize maximal concentrations in urinary excretion has led many authors to formulate needless hypotheses regarding kidney physiology. That the existence of maxima is a common conclusion on the part of the same authors is evident from Cushny's (7, p. 138) generalization that water output is increased whenever any dissolved substance is excreted in large amounts. The importance of the maximal concentrations is shown below in that it serves as an explanation of salt diuresis.

It appears, therefore, that chlorides, bicarbonates, urea, and probably other substances should they attain significant concentrations in the blood, are excreted in solutions of limited total osmotic concentration. There is then a definite constant ratio between solvent and each solute under certain known circumstances, and probably one also between solvent and total solutes.

III. THE DAILY RATE OF WATER EXCRETION. 1. *Urinary water as a measure of catabolism.* In the organism which has received no food or drink for many hours, a condition is reached in which kidney excretions represent net catabolism in the body. The chief of these excretions which leave the body through the kidneys are urea, chloride and water.

During starvation with thirst we expect each to be excreted at a constant rate hour after hour. In actual experiment this constancy is found, and it represents endogenous production. Various modifications of this basal rate of catabolic excretion will be introduced by various regimes, as exemplified in the average figures of table 2.

Upon this basal catabolism was usually superimposed the influence of meals in the experiments which follow. A meal of water was not kept in the body, provided the body tissues were already furnished with water *ad libitum* (16). Protein food in any quantity gave rise to a subsequent excretion of urea which was remarkably regular in its course; the curves obtained upon myself have been given in another paper (18). It is

TABLE 2
Average rates of urinary excretion under various metabolic conditions

	WATER, CUBIC CENTIMETERS PER HOUR	UREA, GRAMS N PER HOUR	CHLORIDE, GRAMS Cl PER HOUR
Starvation without thirst, Benedict's (12) subject L.....	29	0.37	0.01
Regular diet. Before breakfast. E. F. A.....	30	0.33	0.30
Thirst. Third day. E. F. A.....	20	0.26	0.10
Cl-free and N-free diet. Third day. E. F. A.....	42	0.20	0.07

possible, therefore, to calculate the excretion of urea due to a meal during any hour after the ingestion of a known or standard quantity of protein.

Similar curves of excretion following meals were found for chlorides (shown in fig. 6), and have been obtained by other authors for uric acid and other substances (13). These substances, however, are obtained only in minute amounts in the basal condition of nitrogen starvation. The normal curves for water excretion are shown in figure 7. The excretion of both water and chlorides is diminished during the immediate period of digestive activity which follows an ordinary meal.

2. *Obligatory water excretion.* During starvation and during thirst, however, it may be noted that urea and chlorides were always in solutions of such concentration as to approach the maximum characteristic of the kidneys. In other words, the water in the urine under such circumstances did not represent catabolic rate of water production, but was merely that necessary for the carrying of urea and other substances

through the kidneys. Ambard (10) refers to this as "obligatory" water of excretion. This conclusion is of prime importance; the only circumstance under which water was excreted, beyond that necessary to serve as solvent, was after its ingestion in amounts greater than the body will naturally retain.

An illustration of this characteristic of the kidneys rarely to excrete water for its own sake, is found in their contrast to the sweat glands. During exposures to high temperatures sweat is produced which is of a fairly constant dilute composition (14), (15). The object of sweat

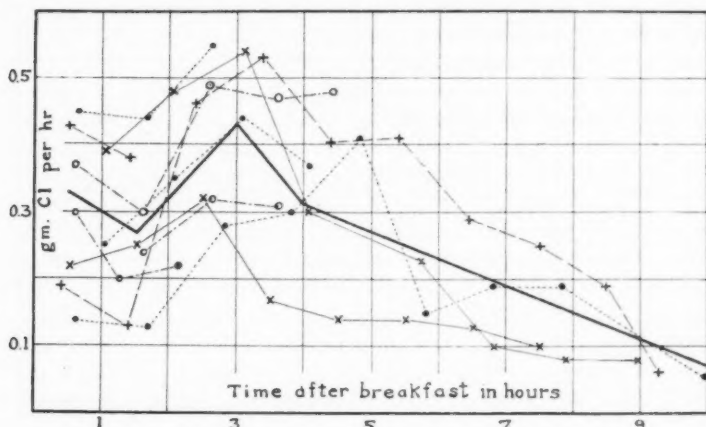


Fig. 6. The rate of chloride excretion on ten normal days. The modal curve was used in calculating the excessive excretion of chloride on days when chloride was ingested, raising or lowering the curve to suit the value for the first hour on the experimental day.

glands is to excrete water without incurring loss of other substances, ordinarily with the consequence that this water will evaporate and thus cool the body surface. The object of the kidneys in terrestrial animals is to excrete the substances without loss of water, and this I have found them ordinarily to do.

It may be stated as a general characteristic of urinary secretion, therefore, that it is the total concentration of dissolved substances which is constant, and not the rate of output of these substances nor the rate of output of any one of them, even water.

This, incidentally, explains the phenomena which have been occasionally pointed out, and most recently by Chaussin (8), of the "antagonism" between the concentrations of chlorides and of urea. Since the total concentration tends to be constant, a low concentration of one of these chief constituents of the urine will naturally be accompanied by a high concentration of the other.

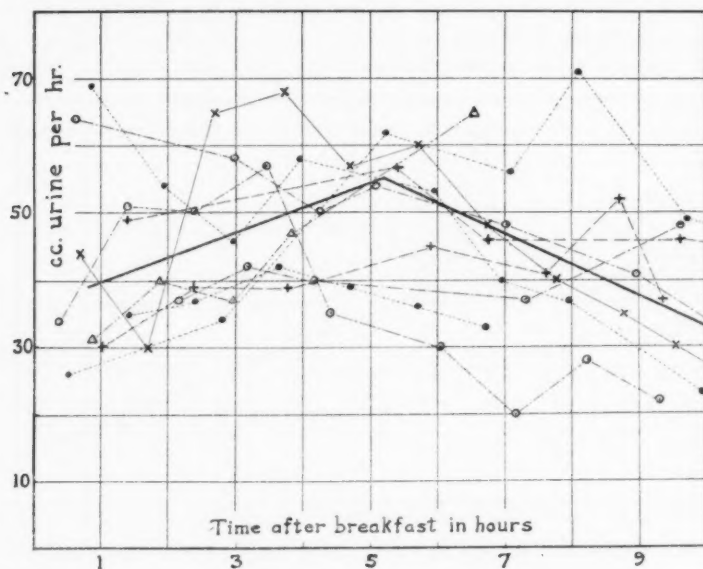


Fig. 7. The rate of water excretion on ten normal days. The modal curve was used in calculating the excessive excretion of water during experiments. It will be noted that the curve of daily water excretion resembles the curve representing total excretion of chloride plus urea, i.e., the total concentration of solutes tends to be constant.

3. *Volume regulation.* The relationships between the kidneys and the volume of the organism are more complex than has been commonly supposed. In metabolic studies no ingesta can be completely accounted for in the excreta; there is always some tucking away, which lasts, after diuresis is past, for many days. That the substances are actually tucked away in the body (and of course water accompanies each of them) is shown most strikingly by the fact that successive ingestions, as of urea

(experiments 18 and 19), do not lead to the total excretion of the later ones; the reserves are always capable of enlargement. This is the experimental difficulty which students of metabolism have never been able to solve. The actual "buffers" to the immediate excretion of ingesta are unknown; for substances which undergo no change in metabolism these "buffers" must be purely physical. Buffer mechanisms, however, are well known to be among the most important properties of living matter; they represent its resistance to sudden influences.

The best exposition of the problem of volume regulation is presented by a protozoan such as *Paramecium*. It regulates its volume by excreting in contractile vacuoles the water which leaks into its protoplasm. Isotonic salt solutions, however, it does not excrete (34), for their water does not leak into its protoplasm because the salts cannot get in. If the isotonic solutions are of such a nature that they will penetrate, as urea will, then the animal should continue to form contractile vacuoles indefinitely, if this view is correct. No opportunity to try this experiment has come to hand.

If one attempts to picture the entire metabolism of water in the human body one soon leaves the realm where exact data are available. It is probable, however, that during starvation, at least, all the water excreted is derived from catabolic processes in cells. While the same water may be reutilized in other cells, some is always in excess. As urea, chlorides and other catabolic products are disposed of, water becomes superfluous, and any of it excreted with less than its maximal concentration of solutes is evidently excreted only because it is superfluous. Indeed, it is only rarely that the rate of water excretion represents rate of catabolism.

Another proof that the water in urine is only that needed for the excretion of dissolved substances is that in extreme thirst (16) the urinary volume is not nearly so low as in sweating (15); in the latter case the solutes are partly eliminated through the sweat glands.

The relative inconstancy with which water is excreted, at times, indicates that its excretion is not primarily controlled by catabolism. This is contrary, on this point, to the conclusion of Oehme (36). The use of water excretion as an index to rate of catabolism is legitimate only when these limitations are recognized. Furthermore, it is the poorest index of all the excreta because, more than any of them, water is metabolized in many ways and can be used over and over again.

4. *The rôle of catabolism in water excretion.* That water catabolism is occasionally a limiting factor in water excretion is shown by two facts.

First, during thirst the character of salt diuresis changes because less water is available to the kidneys, as I have previously shown (16). Second, during sweating the urinary output of water greatly diminishes, even when the body has been previously well supplied with it. The water is evidently not available to the kidneys except as required for the excretion of solutes. A typical experiment is shown in figure 13. The urinary volume during sweating in one experiment fell to 9 cc. per hour.

But often an excess of chlorides and occasionally also of bicarbonates calls for excretion at such a time. The chlorides are evidently left behind by the extraction of water from the blood by the sweat glands, since the average concentration of chlorides in sweat (0.070 M) is less than their concentration in blood (0.100 M). After sweating has ceased there is still a further fall in urinary water, and chlorides evidently no longer demand excretion.

The generalization of authors such as Rowntree (33) that "the amount of urine excreted is directly dependent on the water intake and inversely proportional to the amount excreted by other channels of water loss," is empirically true under ordinary conditions, but does not represent the physiological mechanisms in their true light. The water intake increases the urine volume only after the body is saturated; and the effect of the other channels is through their relieving the kidneys of the excretion of dissolved substances; it is not a direct one.

The limiting factor in the two cases of thirst and sweating is the amount of water which the tissues liberate. This seems to be the only recognizable factor in water excretion outside of the kidneys.

IV. THE CONCENTRATION OF WATER IN NORMAL URINE. If we plot the concentration of chloride found in urine excreted under "normal" circumstances, against the rate of water excretion, we obtain the distribution of points shown in figure 8.

In considering the data for chloride concentration in urine, correction has always been made for the isotonic coefficient or ionization constant (30) upon the assumption that all of the chloride represents sodium chloride. It will be noted that none of the concentrations are above 0.275 M (or 0.490 M corrected for ionization). The only exceptions to this rule, found so far, occurred in the conditions of thirst (16) and occasionally of profuse sweating (15). When the concentrations of urea, however, are plotted against the rate of water excretion, a distribution of points is obtained which is quite different (fig. 9). Here the concentration of urea tends to diminish as the water excretion rises. In general, therefore, "obligatory water" (10) is obligated by

chlorides more than by urea, as was found in the previous study of concentration-maxima. The sum of the concentrations of urea and chlorides (fig. 8), and the values for depression of the freezing point, show that the high urea concentrations often accompany the lower chloride concentrations, and vice versa. The sum, therefore, tends to be constant for all rates of water excretion.

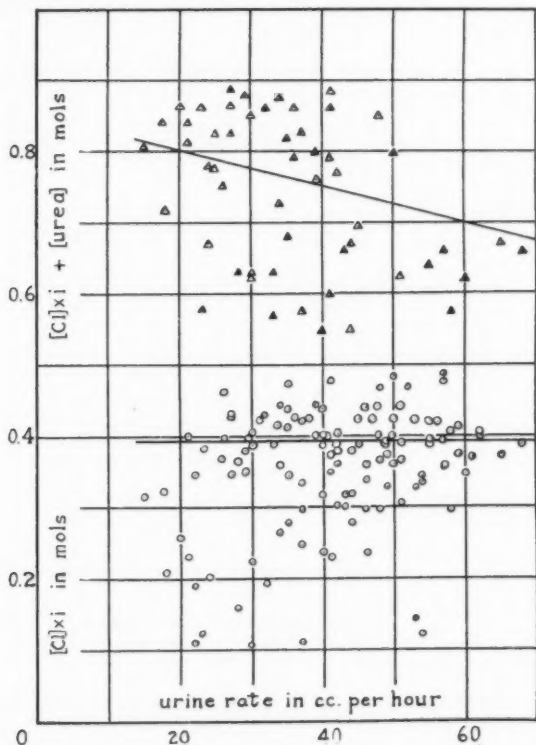


Fig. 8. Below, the concentrations of chloride in urine on normal days compared with the rate of water excretion. Above, the total concentrations of chloride plus urea. The modal curve for the latter points represents a mean between the modal curves for the constituents.

The meaning of these facts with regard to normal urine is not to be sought in some single factor of excretion. Probably the whole body is concerned in the net result (36); but it seems certain that the kidneys

act as gateways, which allow a definite limited quantity of solute in each unit volume of water which passes through; and definitely excrete water in sufficient quantity to get rid of the solutes at the least expense in water.

These two graphs also call attention to the fact that ordinary concentrations tend to be maximal concentrations, which fact helps greatly in the understanding of the normal excretion of water by the kidneys (7, p. 138), (8).

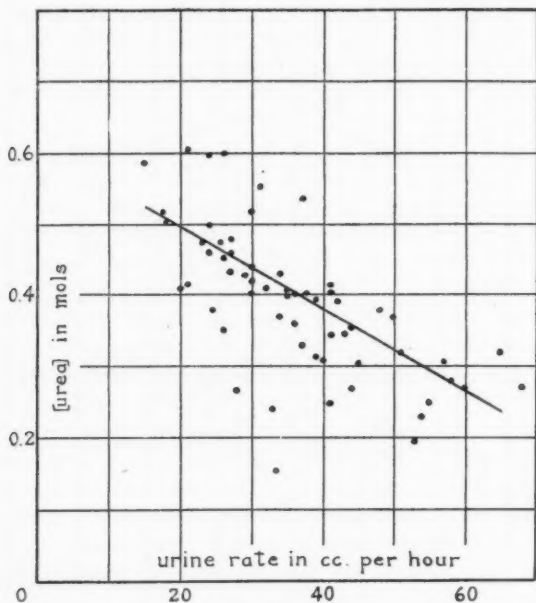


Fig. 9. The concentration of urea in urine compared with the rate of water excretion on normal days.

V. THE STIMULI TO DIURESIS. Diuresis is understood to mean a temporary increase in the rate of water excretion by the kidneys. As has been previously shown, it is an accurate index to the water balance of the body (16) and a useful index to numerous changes in blood composition (17). I have found that the presence in unusual amounts of almost any diffusible substance in the body produces marked changes in the rate of water excretion.

It is useful to distinguish two kinds of diuresis: that due to introduction into the blood of considerable amounts of dissolved substances, and that due to ingestion of water. These may be termed salt diuresis and water diuresis respectively.

TABLE 3
Time relations in salt diuresis

EXPERIMENT NUMBER	SUBSTANCE INGESTED	AMOUNT INGESTED, GRAMS	TIME OF FIRST INCREASE IN RATE OF WATER EXCRETION, HOURS	TIME OF MAXIMAL RATE OF WATER EXCRETION, HOURS	TIME OF MAXIMAL SALT CONCENTRATION, HOURS	REMARKS
36	NaCl	1.8 Cl	1.3	1.5	1.5	Taken in 2 equal doses 1 hr. apart
39	NaCl	2.4 Cl	1.7	4.0	4.0	
49	NaCl	4.9 Cl	0.6	1.6	1.6	
13	NaCl	6.0 Cl	1.4	2.2	1.5	During water privation
1	NaCl	9.1 Cl	1.2	3.2	3.2	
12	NaCl	9.1 Cl	1.2	3.0	6.0	
4	NaCl	10.5 Cl	1.0	3.3	3.3	
2	NaCl	12.1 Cl	1.1	2.5	3.3	
28	NaCl	17.0 Cl	0.5	2.5	6.5	While lying down
5	NaCl	18.2 Cl	1.0	3.5	5.4	
21	NaCl	18.2 Cl	1.0	2.8	6.7	
7	NH ₄ Cl	10.0 Cl	1.0	3.4	5.5	
31	NaCl	18.2 Cl	1.2	4.5	7.7	
33	NaHCO ₃	29.0 HCO ₃	0.6	4.1	6.2	During water privation Privation continued
38	Urea	2.3 N	1.9	1.9	4.9	
16	Urea	14.0 N	0.8	3.5	11.0	
29	Urea	14.0 N	0.5	1.0	5.0	
18	Urea	14.0 N	1.0	2.0	5.7	
19	Urea	21.0 N	0.9	1.9	9.5	Taken in 3 doses 1 hr. apart
22	NaCl+NaHCO ₃	12.1+20.7	0.5	2.0	5.5	
23	NaCl+urea	8.8+14.0	0.4	1.9	5.0	
30	NaCl+urea	9.1+21.0	0.6	4.3	8.8	
32	NaCl+urea	9.1+11.7	0.8	2.6	6.0	

The questions to be answered with regard to salt diuresis are: *a*, Is the water excretion during diuresis always a means of getting rid of the substances in excess? *b*, Is it aroused by an increase in concentration

of some constituent relative to other substances in the circulating fluid? c, Does the response increase as the excessive amount of substances increases? d, Is it proportional for all substances—proportional to their molecular concentration, osmotic pressure, or antagonistic properties?

1. *The excretion of salt in diuresis.* In the cases of sodium chloride, sodium bicarbonate and urea, the rise in rate of water excretion which followed the ingestion of one or more of these substances in hypertonic solution was invariably accompanied by an increase in the concentration of the same substances in the urine (table 3). Since the concentration which the urine may undergo is limited, the increase in water volume was the only method available for rapidly getting rid of the substances from the body.

When salts of ammonia were ingested, there was practically no increase in the ammonia excretion except in the case of ammonium chloride (18). But the amount of urea excreted increased, and this gave a slight diuresis. Evidently urea and not ammonia was the substance in excess in the blood.

When a large quantity of dextrose or sucrose was ingested there was no diuresis, which indicated that the diuresis produced by other substances was not connected with the changes in blood composition during intestinal absorption, liver activity, etc. Moreover, meals produced no diuresis. The text book statement that sucrose is diuretic is not true, when it is ingested by mouth. There was a small amount of sucrose excreted after the ingestion of 200 grams, but not enough to increase the water excretion measurably.

Table 4 shows that not all of the ingested substance was eliminated during the diuresis, however. Both the height of excessive water excretion and its duration are related to the amount of the substance ingested. The diuretic substances were ingested 1 to 3 hours after breakfast, and a knowledge of the normal excretion due to meals (fig. 6) was used in calculating the excretion of excessive amounts of the diuretic. When less than 0.1 gram molecule was ingested into the subject's body 10 to 20 per cent of the substance was excreted during diuresis. When amounts up to 1.0 gram molecules were ingested 30 to 75 per cent was excreted (table 4). Only in the case of ammonium chloride was all of the substance excreted before diuresis ceased (18).

A decrease in amount of water in the body such as occurred in prolonged thirst did not inhibit the diuretic response, though it diminished the height of water excretion (figs. 8 and 9, table 4).

TABLE 4
Water and salt elimination during salt diuresis

EX- PERI- MENT- NUM- BER	SUBSTANCE INGESTED	AMOUNT INGESTED, GRAMS	MAXIMAL RATE OF WATER EXCRE- TION, CUBIC CENTI- METERS PER HOUR	DURA- TION OF EXCESSIVE WATER EXCRE- TION, HOURS	TOTAL EXCESSIVE WATER EXCRE- TED, CUBIC CENTI- METERS	TOTAL EXCESSIVE SALT EXCRETED, GRAMS	PERCENT- AGE OF INGESTED SALT EX- CRETED, DURING ABNORMAL WATER RATE	REMARKS
36	NaCl	1.8 Cl	52	1.9	20	0.2 Cl	11	
39	NaCl	2.4 Cl	40	4.5	25	0.5 Cl	21	
49	NaCl	4.9 Cl	58	4.7	40	0.5 Cl	11	
12	NaCl	9.1 Cl	85	13.2	380	6.5 Cl	71	
4	NaCl	10.5 Cl	138	5.3	320	3.0 Cl	29	
2	NaCl	12.1 Cl	97	10.0	190	3.9 Cl	32	
28	NaCl	17.0 Cl	121	11.1	350	7.2 Cl	42	
5	NaCl	18.2 Cl	179	11.1	80	9.4 Cl	52	
21	NaCl	18.2 Cl	270	7.4		10.3 Cl	57	
31	NaCl	18.2 Cl	191	8.0	570	9.5 Cl	52	
7	NH ₄ Cl	10.0 Cl	213	13.5	950	12.6 Cl	126	
33	NaHCO ₃	29.0 HCO ₃	159	7.7	610	17.6 HCO ₃	61	
38	Urea	2.3 N	52	2.9	20	0.3 N	13	
16	Urea	14.0 N	131	13.5	600	10.5 N	75	
29	Urea	14.0 N	169	5.5	250	6.0 N	43	
18	Urea	14.0 N	72	23.4	470	8.2 N	59	
19	Urea	21.0 N	124	14.4	470	11.2 N	53	
22	NaCl + NaHCO ₃	12.1 + 20.7	281	9.0	1100	4.8 + 13.2	40 + 64	
23	NaCl + urea	8.8 + 14.0	205	14.4	350	6.5 + 10.7	74 + 76	
30	NaCl + urea	9.1 + 21.0	158	8.8	590	3.6 + 12.2	40 + 58	
32	NaCl + urea	9.1 + 11.7	129	11.3	400	5.3 + 58.4	58 + 72	

During water privation

While lying down

During water privation
Privation continued

Evidently the diuresis is not a temporary defense of the body; it is aroused by all changes in blood concentrations. The maximum water output often slightly precedes the maximum salt output; water is mobilized more readily at first. Its efficiency is rarely 100 per cent; the body is able to dispose of the remainder more slowly without extraordinary kidney activity.

The urinary concentration of the substance is unaffected by the amount of substance ingested above a certain minimum, except that the concentration always increases slightly during diuresis. The longest diuresis will, therefore, attain the greatest concentration at its close. The practical identity of the maximal concentration in all chloride diureses is shown in figure 5.

The digestive activities which follow an ordinary meal are accompanied by a diminution or cessation of an experimental diuresis for a period of 1 to 2 hours.

2. *The relation of diuresis to changes in the blood.* The diuresis which follows ingestion of some diffusible substance is always accompanied by an increased excretion of that substance which is present in the body in suddenly increased amounts. The time relations, as shown in table 3, indicate that the first augmentation in secretion of water is always accompanied by the first increase in excretion of salt, and usually by an increase in the concentration of this salt. Sufficient data are at hand to show that all other urinary constituents excreted during diuresis are diminished in concentration; and that their rate of excretion is usually not increased, or only slightly so (7), (19).

Table 3 also shows that the time of maximal water output is nearly the time of maximal salt output. This indicates too that it is the salt content of the blood which excites an increase in the rate of urine production; i.e., of water excretion.

Under definite conditions there is probably an unvarying rate for the excretion of each of the urinary constituents, as suggested above. Such conditions can never be realized completely. By comparison with a large mass of "normal" data, it has been possible to determine exactly how long an excessive rate of salt excretion is maintained. This duration is always as long as, and usually longer than, the duration of excessive excretion of water shown in table 4.

During salt diuresis, measurements of the hemoglobin concentration of the blood were made with the Haldane hemoglobinometer (20). No significant dilution or concentration of this constituent could be detected in the blood from the capillaries of the skin. The concentration

changes in blood to which the kidneys respond by salt diuresis probably do not include changes in the water concentration of the blood. The increases in blood chloride after chloride ingestion (21) and of urea after urea ingestion, are well known. These changes are equally evident whether referred to water or to hemoglobin, therefore.

3. *The qualitative character of the diuretic response* was not found to vary greatly with the quantity of diuretic substance ingested. A series

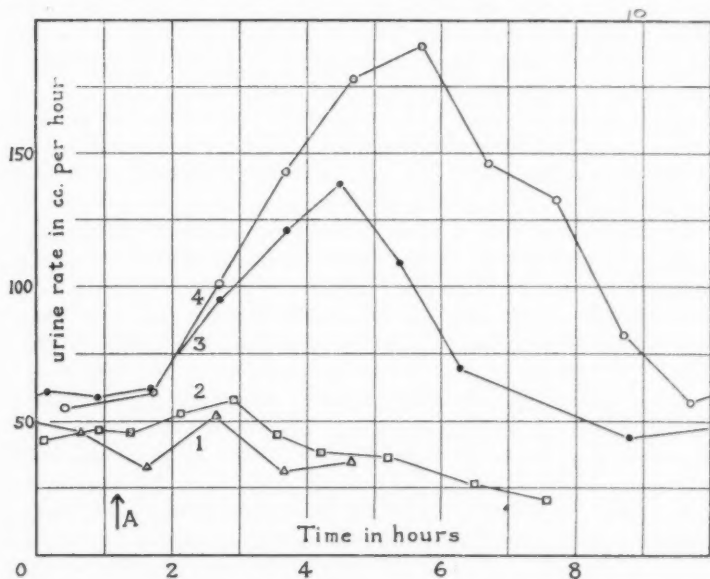


Fig. 10. A comparison of the quality and quantity of diuresis following the ingestion at time A of varying amounts of NaCl. 1. Experiment 36, 3 grams. 2. Experiment 49, 8 grams. 3. Experiment 4, 17 grams. 4. Experiment 31, 30 grams.

of curves which illustrate this point has been superimposed in figure 10. Any slight variation is in the greater duration of the diuresis rather than in its proportional height. It will be seen that the curves differ essentially only quantitatively, and that not even the time of initiation of the response shows any measurable variation.

There are, therefore, two components to the diuresis; the height or intensity of the rate of water excretion, and the length of time during

which an excessive rate continues. Since the concentration of the diuretic substance in the urine is nearly constant during the diuresis which it excites, the product of these two components gives a measure of the amount of substance eliminated, and a formula could easily be derived expressing the relationship. The relationship is not one of simple proportionality because of two factors; first, as already mentioned, the percentage of the dose eliminated is greater with larger doses; and second, as shown above, the maximum concentration increases slightly as diuresis is prolonged. When the diuretic is ingested in more than one dose, the later ones have less effect in increasing the height of diuresis than if the total quantity is ingested at one time.

4. *The quantitative nature of the response.* If we compare the character of the diureses following the ingestion of *different* substances, we find no qualitative differences in the curve of water excretion. Moreover, all hypertonic solutions produced the water excretion as a kidney response in equal amounts of time. It becomes desirable, therefore, to compare the amounts of diuresis called forth by different substances. The diureses produced by chloride ingestion and by urea ingestion may be compared by determining the areas under the curves of water excretion following these ingestions. The results of such comparison are given in table 4, where the areas under the curves of water excretion are expressed as total excessive water output. Averaging these results it turns out that the water output during a diuresis due to chloride is proportional to that due to urea, provided we correct for the ionization of the former. It is, therefore, the osmotic pressure of the diuretic salt which counts. Similarly the maximal rates of water excretion, plotted in figure 11, indicate that water excretion is excited directly by the number of solute particles to be eliminated by the kidneys, regardless of their chemical character.

Diuresis is caused also by excess of acids or alkalis. I have found that in all such cases a maximal concentration of either acid salts or of bicarbonates is attained. In acidosis it is not the hydrogen ions as such which concern the kidneys, but the acid salts which are mobilized for their excretion. Bicarbonate diuresis is illustrated by the water excretions during forced breathing, during heat-sweating and after bicarbonate ingestion (figs. 3 and 13).

The conclusion seems to be warranted from these considerations that diuresis is the same for any diffusible substance introduced into the blood, and proportional to the number of dissolved particles introduced. Evidently no salts or acids or alkalis or simple organic compounds can

be regarded as specific diuretics, as is done in some text-books. These substances produce diuresis by the fact that the kidneys excrete them all more rapidly than increase in concentration alone would allow.

VI. THE CHEMICAL CONTROL OF KIDNEY ACTIVITIES. Is it possible to distinguish what factors arouse similar responses in the kidneys' water output, and from this to deduce what the stimuli are?

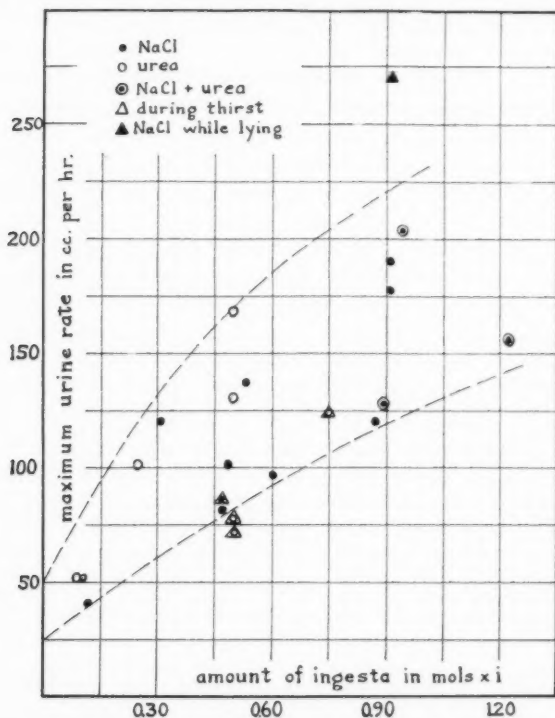


Fig. 11. The influence of amount of ingested NaCl and urea upon the maximum rate of diuresis. All chloride values are corrected for ionization in 0.3 M solution. The peculiarity connected with lying down has been explained in a previous paper (16).

1. *Water diuresis.* Let us take as a starting point the excretion of excessive water. This water diuresis was distinctly aroused by the ingestion, when the body was in water equilibrium or "saturation," of as little as 150 cc. of water. This is about 2 cc. per kilo, one-fifth

of the least detectable amount as found by Hashimoto (45). Table 5 indicates the results of several experiments in which larger quantities of water were ingested. In the case of the present subject a maximal excretory rate of about 900 cc. per hour was found. If water were drunk at a rate faster than this, a plethora would evidently be reached in the body. The limiting activity, however, is probably that of intestinal absorption, so that the kidneys' limit was not found. Priestley (23) on himself reached 1200 cc. per hour.

TABLE 5
Time relations and water elimination during water diuresis

EXPERIMENT NUMBER	SOLUTION INGESTED	AMOUNT INGESTED, CUBIC CENTIMETERS	DURATION OF INGESTION, HOURS	TIME OF FIRST INCREASE IN RATE OF WATER EXCRETION, HOURS	TIME OF MAXIMAL RATE OF WATER EXCRETION, HOURS	DURATION OF EXCESSIVE WATER EXCRETION, HOURS	TOTAL EXCESSIVE WATER EXCRETED, CUBIC CENTIMETERS	MAXIMAL RATE OF WATER EXCRETION, CUBIC CENTIMETERS PER HOUR
3	Water	1500	2.0	1.0	2.0	6.5	1470	790
9	Water	2500	5.0	1.0	3.8	7.0	2470	920
24	Ringer (23)	2000	0.9	1.0	1.5	23.0	300	356
44	1.0 per cent NaCl	1000	0.2	2.0	2.6	3.1	10	41
15	1.0 per cent NaCl	1000	1.0	1.0	4.2			111
17	1.0 per cent NaCl	2000	5.5	1.5	5.0	44.0		102
25	1.0 per cent NaCl	3000	1.0	1.0	6.0	46.0		299
42	Locke	1000	0.1	1.0	2.0	4.5	80	49
48	1.7 per cent NaBr	500	0.1	0.9	1.0	0.7	30	75
43	1.3 per cent KCl	500	0.1	1.0	1.9	4.0	420	229
46	1.3 per cent KCl	500	0.1	0.6	1.2	2.6	200	113
40	1.8 per cent urea	250	0.1	1.0	1.0	2.0	120	132
45	1.7 per cent CaCl ₂	500	0.1	0.7	0.7	2.3	-20	-13

The efficiency of the kidneys in adjusting their activities to water excretion is very great, for these limiting rates are 20 to 30 times those at which the kidneys usually excrete water. As far as is known, these high rates can be kept up indefinitely.

2. *Blood dilution and water diuresis.* It has been supposed by Haldane and Priestley (24) that the kidneys must be responding to blood dilution in water diuresis. They have been able to find a slight increase in the water concentration of the blood relative to its electrolytes (25), but not relative to its hemoglobin content. It seems that the evidence is not complete (26), (39) that the kidneys take the water from the blood

in order to adjust its osmotic pressure or diffusion pressure. That absorption is a chief factor in this water diuresis is indicated by the well-known fact that intravenously injected water does not give diuresis (41), (26).

It occurred to me that the ingestion of isotonic solutions might offer a crucial experimental test. Priestley (23) ingested a Ringer's solution in some of his experiments, but this I found (16, fig. 2) to be distinctly hypotonic to human blood. Upon ingesting large amounts of 1 per cent NaCl solution, I was surprised to find practically no diuresis (16). The solution was absorbed readily and held in the body. This has since been confirmed by Baird and J. B. S. Haldane (27). Locke's solution was treated in the same way, and likewise a 1.7 per cent solution of sodium bromide. Evidently solutions which produced no change in the electrolyte composition of the blood did not arouse the kidneys to excrete water.

The results with isotonic solutions are given in table 5. In no case did the ingestion by mouth of such a solution lead to the excretion of more water than was ingested, as injected solutions were found to do by Thompson (41).

When isotonic potassium chloride was ingested a different result from that with sodium salts was obtained. Five hundred cubic centimeters produced a very rapid and high diuresis, and practically all of the ingested fluid and its solutes was immediately gotten out of the body (fig. 12). Two hundred and fifty cubic centimeters of an isotonic solution of urea produced a similar result. Evidently there is no confusion on the part of the kidneys between NaCl and KCl; potassium was treated just like urea—a substance calling for immediate excretion. As far as the phenomena of absorption are known, there is no way in which an ingestion of isotonic KCl could produce a diffusion pressure in the blood different from that which isotonic NaCl would produce. While potassium is known to have a threshold in the plasma (7), the threshold is so low that an isotonic solution would give a great excess. This seems to be the fact of the distinction between KCl and NaCl which the kidneys made in the present experiments; though according to Boek (35) and Miller (40), this distinction cannot be laid to the kidneys, since the potassium displaces sodium, in part, in the body. Injected isotonic KCl likewise gives diuresis (35).

An alternative explanation is that the kidneys respond to any change in the relative proportions of the blood constituents. Immediately the question arises: to what do the kidneys compare each concentration?

Water diuresis indicates that the proportions are not relative to water concentration; lack of diuresis after ingesting Locke's solution indicates that the proportions are not relative to the non-diffusible substances of the blood except over long periods. There is no possibility, therefore,

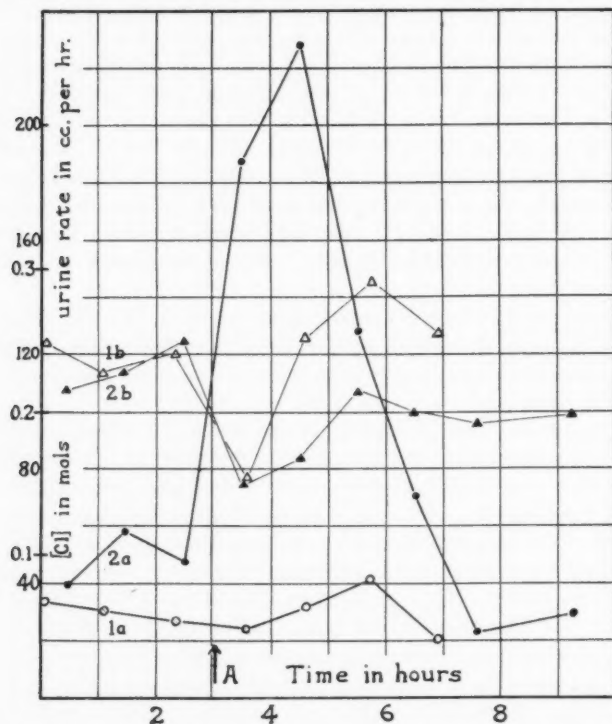


Fig. 12. Comparison of the urinary excretions after ingesting isotonic NaCl and after ingesting isotonic KCl at time A.

1. Experiment 44, ingested 1000 cc. of 1.0 per cent NaCl; 1-a, rate of urine excretion; 1-b, chloride concentration.

2. Experiment 43, ingested 500 cc. of 1.3 per cent KCl; 2-a, rate of urine excretion; 2-b, chloride concentration. Chloride concentrations uncorrected for ionization.

that the kidney's responses are referable to any single factor in blood composition. They appear to exhibit a definite response to almost any change in the equilibria of the blood.

In another experiment isotonic calcium chloride (1.3 per cent) was ingested. Five hundred cubic centimeters of it gave no diuresis, and in addition it inhibited the normal excretion of urinary constituents for a definite period. This inhibition has the exact character of a negative diuresis.

3. *The relation of blood electrolytes to the kidneys.* To what, then, do the kidneys respond? It is well known (45), (28) that intestinal absorption of pure water involves the initial diffusion of electrolytes into it from the blood tissues. Water diuresis is, perhaps at its beginning, due to the extraction of solutes from the blood, which involves a change in diffusion pressure relative to the diffusible substances, but not relative to the non-diffusible content of the blood. Isotonic sodium chloride solutions when added to blood do not change the diffusion pressure of the blood, but do change the concentration of non-diffusible substances. Isotonic potassium chloride solutions do the same thing physically, and yet cause diuresis. The effect of isotonic calcium chloride solutions may possibly be counted as a change in the opposite direction; calcium is well known as an inhibitor of permeability. This factor of salt antagonism is a real one in the case of the kidneys, and has not been hitherto sufficiently recognized. Nevertheless the facts can still be accounted for upon the threshold idea.

Since the concentration of chloride in the blood does not change during a diuresis due to isotonic KCl, it is impossible to explain it entirely as a salt diuresis. It is probably a simultaneous salt diuresis and water diuresis. The salt KCl is excreted, the water which would have made it isotonic evidently becomes superfluous, and it is excreted by the kidneys. One might possibly expect the Cl ions to hold back the K ions, but they do not.

The striking phenomenon, however, is not that isotonic KCl is excreted rapidly, but that isotonic NaCl is not excreted rapidly. Were the kidneys concerned merely with thresholds, then the excessive NaCl would be excreted just as rapidly as KCl, according to Ambard's law of the secretory constant. NaCl is excreted in the predicted manner when it is ingested in hypertonic solution, but not in isotonic. Possibly our conception of thresholds must be modified by one of increments. A very small amount of ingested KCl is an infinite increment of K in plasma, for plasma normally contains very little. But it contains about 0.10 M sodium, and any measurable increment is detected if the concentration of sodium increases, but not if the isotonic solution is added to the blood as such.

If we try to indicate what substances the kidneys recognize as chemical individuals, we find interesting data, but few of real significance. Cushny (7) has catalogued them. Ambard (1) mentions the fact that urea ingestion seems to augment the chloride excretion, indicating a replacement of chlorides by urea in the tissues. The evidence for this is not particularly conclusive, and I have not noticed it in any of my own numerous experiments. I did find evidence (experiment 6) that bicarbonate ingestion pushed chlorides from the body, and from the above

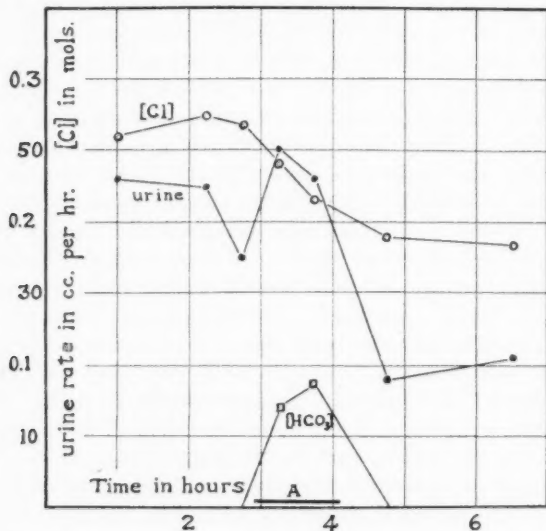


Fig. 13. Urine excretion during sweating. Experiment 50. Sweating for 72 minutes at A; 103°F., 100 per cent relative humidity, no air motion. Rate of urine excretion proportional to total concentration of chloride and bicarbonate. Chloride concentrations uncorrected for ionization.

studies it is evident that the two are in one respect identical to the kidneys, though perhaps not to the body as a whole. The ingestion of isotonic sodium bromides showed that it was not distinguished by the body from sodium chloride. The data of Smith and Mendel (31) on injection of isotonic solutions and the restoration of blood volume made it less necessary to try out a large number of other substances with regard to the kidneys' powers of discrimination.

There is nothing in the present data to justify objection to the hypothesis that water diuresis is in response to blood dilution. Every substance is excreted in proportion to its concentration above the threshold, Ambard has shown. There must be a water threshold, too. Values above this are equivalent to an excessive diffusion pressure.

4. *Regulation of the blood composition.* The kidneys are evidently the primary means for regulating the composition of the blood as regards its non-volatile, diffusible constituents. The so-called thresholds for each substance are maintained with remarkable constancy. But the kidneys are dealing in reality with equilibria and increments, not with overflowing thresholds. Upon this view it is possible to conceive why it is so hard to divert the kidneys from their work of maintaining the blood composition.

The kidneys apparently are not primary regulators of the blood volume. This is a much slower adjustment than that of blood composition—it is the factor which comes into play after the ingestion of isotonic sodium chloride or Locke's solution. The factors in the adjustment of blood volume are apparently not understood (44), (29), though the factors of vascular tone, heart output, lymph formation and blood formation have been studied separately. I do not believe that the kidneys have primarily to do with this adjustment since, as shown above, their responses are not relative to the amount of any one single constituent of the blood, such as plasma protein, corpuscles, chloride, etc. The ratio of diffusible to non-diffusible substances in the blood is evidently destroyed in the hydremia caused by isotonic NaCl. The lack of diuresis under this condition is not explained by the theories of Knowlton (42), Milroy (43) and Bayliss (44) concerning the regulation of blood composition and volume.

The kidneys are merely the path by which substances are eliminated, after other tissues have converted the volume change into a composition change. This is in general agreement with L. J. Henderson's (32) statement that volume regulation is independent of the excretion of substances in urine.

CONCLUSIONS

1. The human kidneys exhibit a definite limit in their powers of concentrating the urine. This limit is characteristic and identical for each substance which they excrete, such as chloride, phosphate, urea and bicarbonate. There is also another limit to the total concentration of substances in the urine, the mechanisms concerned in its production are not yet clear.

2. The rate at which water is catabolized by the tissues has been found normally not to equal the rate at which the kidneys demand it to aid in the excretion of dissolved substances. It is usually demanded for the excretion of chloride rather than for the excretion of urea; the former is the chief factor in determining the urinary volume.

3. Since the volume of water in urine is constantly proportional to the amount of dissolved substance in it, diuresis is an exact measure of the dissolved substances in blood which have changed the blood's composition from normal. At a given moment diuresis is proportional to the increment above normal which demands excretion; the increment being the excess osmotic concentration of particulate concentration in the blood.

4. The chemical control of the kidney's activities can nearly always be measured by the variations in rate of water excretion. A study of isotonic solutions has shown that their excretion is as reliable a measure of changes in blood composition as certain actual changes in blood measured by other workers. Water excretion, therefore, gives a fairly complete picture of the measurable variations in blood composition. It is not influenced by changes in blood volume or body volume.

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THE INFLUENCE OF COMBINATIONS OF INORGANIC SALTS
AND OF VARIATIONS IN HYDROGEN ION CONCENTRA-
TION ON THE HELIOTROPIC RESPONSE OF ARENICOLA
LARVAE

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Received for publication May 12, 1923

Some years ago, while studying the effects of inorganic salt solutions on the ciliary and muscular movements of *Arenicola* larvae, the senior author found certain definite correlations between the positive heliotropism of these organisms and the nature and proportions of the salts present.¹ Heliotropic swarming ceased instantly in pure isotonic NaCl solution, but persisted for several minutes in a NaCl solution to which a small proportion of CaCl_2 was added. A similar but much less favorable effect was produced by MgCl_2 . If both CaCl_2 and MgCl_2 were added in appropriate proportions, the effect was more favorable than that produced by either salt alone. The addition of a little KCl to the solution still further improved the conditions. Relations of this kind are not unexpected, and are characteristic of salt antagonisms in general. It was also found that in various pure and mixed solutions in which ciliary activity and swimming movements persisted for some time (solutions of MgCl_2 , KCl, CaCl_2 and mixtures of these salts) the larvae showed no orientation to light and swam at random; in these cases closer examination showed that muscular movement was paralyzed or impaired. A means of analyzing the mechanism of the heliotropic response was thus suggested; for example, the absence of orientation in media containing a high proportion of MgCl_2 , in which muscular contraction is prevented while cilia remain almost normally active, indicates that the condition determining the orientation is the influence of light on the musculature rather than on the cilia. This interpretation was confirmed by the observation that larvae which have lost all muscular movement and heliotropic response in Mg-containing solutions quickly recover both on return to sea-water. Anesthetics (ether,

¹ R. S. Lillie: This Journal, 1901, v, 56; 1903, vii, 25.

alcohols, chloroform, etc.) in appropriate concentrations also destroy orientation without evidently altering the rate of the swimming movements;² this effect depends on the relatively high susceptibility of the musculature to narcosis as compared with the cilia. Such facts show that orientation in a light field depends on the balanced action of the musculature on the opposite sides of the body, the cilia simply furnishing the means of propulsion.

A further illustration of the intimate relations between the light reactions of these organisms and the composition of the external medium is seen in the readiness with which the normal positive heliotropism is reversed by the addition of small quantities of foreign substances to the sea-water. In sea-water thus modified a variable and often large proportion of larvae exhibit negative instead of positive heliotropism; mineral and organic acids, bases (NaOH and NH_3), lipoid-solvent compounds in general, cytolytic compounds (saponin, foreign blood sera) all produce this effect. Dilution or concentration of the sea-water or increase in the proportion of NaCl (by dilution with isotonic NaCl solution) also renders the larvae negative. Such larvae recover the normal positive response on return to sea-water.³

EXPERIMENTAL. During the past summer at Woods Hole we have investigated in further detail the relations between the inorganic salt-content of the medium and the heliotropic response of the larvae, using various combinations of salt solutions; we have also studied the effect of varying the H-ion concentration of the balanced artificial solution (containing NaCl, CaCl_2 , MgCl_2 and KCl) in which the light reaction is most nearly normal.

The larvae are readily obtained in large numbers from the egg-strings which are hatched in the laboratory; the freshly emerged larvae are about $\frac{1}{3}$ mm. long and uniform in their reactions to light, swimming rapidly and in almost straight lines toward a window or artificial source. The movement is easily seen without a microscope. In estimating the duration of heliotropism in the different artificial solutions it is desirable to observe many organisms at the same time, and most of our observations were made with the naked eye or simple lens rather than with the compound microscope. Light from a single artificial source was used in preference to daylight; the larvae were viewed against a dark background in watch glasses placed within a dark chamber illuminated from one side. This observation chamber consisted of a shallow card-

² Unpublished observations.

³ Cf. S. Kanda: Biol. Bull., 1919, xxxvi, 149, for a study of the reversal of heliotropism in *Arenicola* larvae and references to the earlier observations.

board box, about 9 inches square, blackened on the inside, into which light was admitted through a narrow slit about $\frac{1}{2}$ inch wide extending along one side. The source of light was a 25-watt tungsten filament lamp placed at about 20 inches distance. The larvae were observed in shallow watch-glasses, *ca.* $2\frac{1}{2}$ inches in diameter, of regular concave form, placed just inside the slit; three of these could be arranged side by side at equal distances from the lamp. The light, entering the solutions at a low angle, was sufficiently intense to orient the larvae rapidly and definitely, so that in sea-water or appropriate salt solutions they all swam rapidly across the field toward the lamp. To permit observation without the admission of light from above, cardboard tubes about 5 inches long and 2 wide were inserted into the box immediately above the watch-glasses; the larvae were observed through these tubes. Practically the only light entering the tubes during an observation is that reflected from the bodies of the larvae. The latter are thus seen distinctly against a dark background.

The experimental procedure was as follows. A small volume of sea-water containing numerous freshly hatched larvae was placed in a watch glass near a source of light, in order to gather the organisms on the light side of the watch glass. The surplus sea-water was then removed with a pipette and blotting paper; the watch-glass was placed in position inside the observation chamber, and the solution was then rapidly added with a large pipette in such a way as to distribute the larvae uniformly in the solution. The duration of the period of heliotropism was then determined, using a stop-watch to measure the shorter intervals. The larvae exhibit remarkable uniformity in the duration of the heliotropic reaction in a solution of definite composition; in those solutions in which heliotropism lasts for only a short time the disappearance of orientation in the majority can be determined within a few seconds. The times given in the tables represent the averages of six experiments with each solution. The separate readings gave closely concordant results.

SALT SOLUTIONS. Stock solutions (2.5 M) of the four chief chlorides of sea-water, NaCl, KCl, CaCl₂, MgCl₂ were standardized by titration. The salts used were good commercial preparations (Kahlbaum's and Baker's analyzed chemicals). The water was distilled a second time in glass. The dilutions regarded as isotonic with sea-water were *m*/2 NaCl and KCl, and *m*/3 CaCl₂ and MgCl₂. To insure uniformity in the H-ion concentration of the solutions the reaction was in all cases brought to the pH of sea-water (*ca.* 8) before using. RbCl and CsCl were also used in a number of experiments for comparison with KCl.

ACTION OF DIFFERENT SALT SOLUTIONS ON HELIOTROPISM: *Solutions of single chlorides.* No heliotropic response was shown in any of the solutions of a single salt. All pure solutions are toxic, but the salts differ characteristically in their action on muscular and on ciliary movement; cilia are rapidly injured and paralyzed in pure NaCl solutions, while in solutions of the other three salts, especially $MgCl_2$ and KCl, they continue activity for some time. Muscular movement, however, is quickly paralyzed in these solutions and the swimming movements lose all orientation, showing that for heliotropic swarming the combined action of both motor elements is necessary.

TABLE I

Approximate average duration of heliotropic swarming in mixtures of m/2 NaCl and m/3 $CaCl_2$

VOLUMES m/2 NaCl	VOLUMES m/3 $CaCl_2$	DURATION OF HELIOTROPISM
99.0	1.0	1 min. 30 sec.
98.0	2.0	2 min. 50 sec.
97.0	3.0	3 min. 0 sec.
95.0	5.0	3 min. 30 sec.
92.5	7.5	4 min. 0 sec.
90.0	10.0	4 min. 20 sec.
88.0	12.0	5 min. 30 sec.
85.0	15.0	5 min. 0 sec.
82.5	17.5	4 min. 15 sec.
80.0	20.0	3 min. 50 sec.
75.0	25.0	3 min. 20 sec.
70.0	30.0	3 min. 0 sec.
60.0	40.0	2 min. 45 sec.
50.0	50.0	2 min. 30 sec.
40.0	60.0	2 min. 25 sec.
30.0	70.0	2 min. 20 sec.
20.0	80.0	2 min.
10.0	90.0	ca. 1 min.
5.0	95.0	ca. 20 sec.

Solutions containing two chlorides. 1. *NaCl and $CaCl_2$.* The average length of time during which the larvae exhibit heliotropic orientation in the various mixtures of m/2 NaCl and m/3 $CaCl_2$ may be seen from table 1. The addition of even so little as 1 cc. m/3 $CaCl_2$ (in 100 cc. of solution) produces a striking change in the action of the NaCl solution; ciliary activity is prolonged and the larvae exhibit heliotropic swimming movements for a minute or more. This favorable effect increases rapidly with further increase in the proportion of $CaCl_2$,

reaching a maximum in mixtures containing 12 to 15 volumes per cent $m/3$ $CaCl_2$; in these solutions heliotropism lasts about five minutes. With further increase in the Ca-Na ratio the duration of the heliotropic response gradually decreases. An early stiffening of muscular movements and a gathering of the larvae in clumps are characteristic effects of solutions containing more than 50 volumes per cent $m/3$ $CaCl_2$; but even in mixtures containing so little as 5 volumes per cent $m/2$ $NaCl$ a brief heliotropic response is seen.

2. *NaCl plus $MgCl_2$.* The addition of magnesium to the $NaCl$ solution has a relatively slight effect as compared with that of calcium in preserving heliotropic orientation. A larger quantity is required to produce a distinct effect; even in solutions containing 10 volumes per cent of $m/3$ $MgCl_2$ heliotropism is only momentary; at the optimum proportions (25 to 30 volumes per cent $m/3$ $MgCl_2$) all orientation dis-

TABLE 2
Duration of heliotropism in mixtures of $m/3$ $CaCl_2$ and $m/2$ KCl

VOLUMES $m/3$ $CaCl_2$	VOLUMES $m/2$ KCl	DURATION OF HELIOTROPISM
96.00	4.00	0
97.00	3.00	ca. 10 sec.
98.00	2.00	35 sec.
99.00	1.00	1 min. 20 sec.
99.25	0.75	1 min.
99.50	0.50	ca. 20 sec.
99.75	0.25	Momentary

appears in less than a minute. This unfavorable action is to be referred to the characteristic anesthetizing or paralyzing influence of the magnesium on muscular contraction; this influence quickly shows itself in solutions containing 30 or more volumes per cent $m/3$ $MgCl_2$.

3. *NaCl plus KCl .* The presence of a small proportion of KCl is favorable to muscular contractility; higher concentrations (above 10 volumes per cent) have a rapid paralytic action; potassium is also favorable to ciliary movement which continues for some time even in the pure $m/2$ KCl . In mixtures of the two chlorides a brief heliotropic response is seen in solutions containing from 2 to 5 volumes per cent $m/2$ KCl , with an optimum at 4 volumes per cent (lasting 1 to 2 minutes). In mixtures containing 10 volumes per cent $m/2$ KCl and higher no heliotropism is seen.

4. *$CaCl_2$ plus KCl .* It is remarkable that even in the entire absence of $NaCl$ the addition of a small quantity of KCl to $m/3$ $CaCl_2$ enables

heliotropism to continue for a short time (table 2). The effect is slight, exhibits an optimum at 1 volume per cent $m/2$ KCl, and disappears at concentrations above 3 per cent.

Solutions containing three chlorides. The favorable relation of potassium to the heliotropic response is shown in a striking manner when $m/2$ KCl is added to solutions containing both NaCl and $CaCl_2$. In the absence of potassium heliotropism soon disappears, even under conditions otherwise favorable, *e.g.*, in solutions containing NaCl, $CaCl_2$ and $MgCl_2$ in balanced proportions. Apparently potassium furnishes some necessary condition for the stability of the mechanism underlying the heliotropic response; this is the more remarkable since even a slight increase above the normal concentration of this element is highly injurious.

TABLE 3

Duration of heliotropic swarming in solutions obtained by adding varying volumes of $m/3$ $MgCl_2$ to 100 volumes of a mixture of 88 volumes $m/2$ NaCl plus 12 volumes $m/3$ $CaCl_2$

VOLUME OF $m/3$ $MgCl_2$	DURATION OF HELIOTROPISM
5	5 min. 30 sec.
10	7 min. 15 sec.
15	10 min. 30 sec.
20	13 min.
25	20 min.
30	17½ min.
35	14½ min.
40	12½ min.
50	3 min.
60	3 min.
70	2 min. 50 sec.
80	2 min.
85	ca. 20 sec.

The presence of sodium and calcium salts in the external medium, with the former greatly in excess, appears to be a fundamental condition for the continuance of normal life-processes in the cells of marine animals and the derived fresh-water and terrestrial forms. Magnesium is of less general importance. In studying the action of solutions containing three chlorides, we have regarded the Na-Ca combination as the basic one, and our procedure has been to add varying quantities of the third salt to the optimum mixture of NaCl and $CaCl_2$ as already determined, *viz.*, 88 volumes $m/2$ NaCl plus 12 volumes $m/3$ $CaCl_2$. For brevity we shall call this the Na-Ca solution.

Na-Ca solution plus $MgCl_2$. The addition of $MgCl_2$ to the Na-Ca solution has only a limited effect in prolonging the heliotropic response, although in other respects, *e.g.*, as regards the possible duration of life in the solution, such a mixture is much more favorable than the simple Na-Ca solution. Table 3 shows the observed duration of heliotropism in the series of solutions employed. To 100 volumes of the Na-Ca solution m/3 $MgCl_2$ was added as indicated. In the most favorable combination, 100 volumes Na-Ca solution plus 25 volumes m/3 $MgCl_2$, heliotropic swimming lasts about 20 minutes; further addition of $MgCl_2$ interferes rapidly with muscular movement; this effect increases most rapidly in the interval between 40 and 50 volumes m/3 $MgCl_2$, and soon abolishes the heliotropic response. Comparison with the Na-Mg solutions shows that calcium antagonizes strongly the inhibitory action of

TABLE 4
Duration of heliotropism in solutions obtained by adding varying volumes of m/2 KCl to 100 volumes Na-Ca solution

VOLUMES m/2 KCl ADDED	DURATION OF HELIOTROPISM
1	ca. 25 min.
2	ca. 43 min.
3	Some hours
4	Some hours
5	ca. 4½ min.
7	1 min. 45 sec.
10	Momentary

magnesium on muscular contraction; but the presence of magnesium, although favoring the continuance of heliotropism, does not furnish the conditions required for prolonging the latter indefinitely.

Na-Ca solution plus KCl. These conditions are, however, furnished by the presence of a comparatively small quantity of KCl. The addition of only 1 volume m/2 KCl to 100 volumes of the Na-Ca solution produces a medium in which heliotropism lasts longer than in the optimum Na-Ca-Mg solution (table 4). In solutions to which 3 and 4 volumes m/2 KCl were added an apparently normal heliotropism persisted for many hours. The concentration of KCl producing this effect is definite and low, and similar to the concentration of KCl in sea-water. Increase of potassium above this critical concentration is rapidly unfavorable; the addition of 5 volumes m/2 KCl shortens the period of heliotropism to less than 5 minutes; the addition of 10 volumes m/2 KCl produces muscular incoördination and the response ceases at once.

Rubidium is a very effective substitute for potassium, but caesium is relatively unfavorable. The contrast between the two metals is shown in tables 5 and 6. The addition of 2 cc. m/2 RbCl to 100 cc. of the Na-Ca solution produces a medium in which normal heliotropism lasts for many hours. Further addition represses heliotropism and produces muscular paralysis, as in the case of KCl, although the effective concentrations are lower. In the case of CsCl the favorable effect of smaller additions (1 to 3 cc. m/2 CsCl) is slight and the interference with heliotropism resulting from larger additions is less pronounced than with either RbCl or KCl.

TABLE 5

VOLUMES m/2 RbCl ADDED TO 100 VOLUMES Na-Ca SOLUTION	DURATION OF HELIOTROPISM
0.25	6 min.
0.50	6 min. +
1.00	15 min. +
2.00	Some hours
3.00	8 min.
4.00	ca. 3 min.
5.00	ca. 10 sec.

TABLE 6

VOLUMES m/2 CsCl ADDED TO 100 VOLUMES Na-Ca SOLUTION	DURATION OF HELIOTROPISM
1	ca. 10 min.
3	7 min. +
5	5 min.
7	4 min. 50 sec.
10	4 min.
12	3 min. 10 sec.
15	2 min. 15 sec.
20	1 min.
30	ca. 30 sec.
35	Momentary

The ability of rubidium to replace potassium as a constituent of physiological media is well known; RbCl is a highly favorable substitute for KCl in maintaining the beat of frogs' hearts perfused with Ringer's solution and in its action on mammalian smooth muscle;⁴ also in pro-

⁴ A. J. Clark: Journ. Pharm. Exper. Therap., 1922, xviii, 423.

moting the development of sea-urchin eggs in artificial sea-water,⁵ and in enabling cell-division and embryo-formation in *Fundulus* eggs to continue in pure solutions of various salts (CaCl_2 , Na-citrate, LiCl).⁶ Whether, as suggested by Zwaardemaker,⁷ this similarity of action has any relation to the slight radioactivity possessed by this element in common with potassium is uncertain. Experiments by the authors just cited^{4, 5, 6} lend little support to this idea. We have not yet tested the action of radioactive elements (uranium, thorium) in maintaining the heliotropism of *Arenicola*. On the other hand, in the experiments of Clark and of J. and R. Loeb, as well as in our own with *Arenicola* larvae, caesium proved to be a much less favorable substitute for potassium than rubidium. Höber's experiments indicate that in its general physiological action caesium is more closely related to sodium and lithium than to potassium.⁸

Na-Ca solution plus MgCl_2 and KCl. The physiological deficiency in solution 5 of table 3 (containing NaCl , CaCl_2 and MgCl_2 in the most favorable proportions) seems to be completely removed by the addition of a small proportion of KCl. When 4 volumes m/2 KCl were added to 100 of this solution a medium resulted in which heliotropic response persisted for more than two days. This medium, in its relation to the heliotropic reactions of the larvae, is apparently an almost perfect substitute for sea-water. The combination of CaCl_2 and MgCl_2 apparently antagonizes the paralytic action of the KCl more completely than does CaCl_2 alone; thus heliotropism lasted some hours in a solution to which 6 volumes m/2 KCl were added, although in the Na-Ca solution without Mg the addition of 5 volumes shortened the response to a few minutes. With the addition of 12 volumes m/2 KCl to the Na-Ca-Mg solution heliotropism disappeared in a few seconds.

INFLUENCE OF VARIATION OF H-ION CONCENTRATION. The normal H-ion concentration of the sea-water in which the larvae were hatched was measured by the hydrogen electrode method and found to vary between 2×10^{-8} and 2×10^{-9} (pH 7.7-8.7). In studying the effects of varying H-ion concentrations the above balanced medium containing

⁵ Herbst: Arch. f. Entwicklungsmech., 1901, xi, 617; Robert Loeb: Journ. Gen. Physiol., 1920, iii, 229.

⁶ J. Loeb: Journ. Gen. Physiol., 1920, iii, 237.

⁷ Zwaardemaker: Journ. Physiol., 1919-20, liii, 273.

⁸ Cf. Höber: Physik. Chem. d. Zelle u. d. Gewebe, 4th ed., 1914, Chapt. 10; Höber and Waldenberg: Arch. gesamt. Physiol., 1909, cxxvi, 331.

⁹ R. S. Lillie: This Journal, 1909, xxiv, 14; cf. footnote, p. 35.

the four chlorides was used, *viz.*, 88 volumes m/2 NaCl *plus* 12 volumes m/3 CaCl₂ *plus* 25 volumes m/3 MgCl₂ *plus* 4 volumes m/2 KCl. The H-ion concentration was increased by adding dilute HCl and decreased by dilute NaOH (and KOH). The indicator method was used as far as possible in determining the pH, although for the higher alkaline range it was considered more satisfactory to use the hydrogen electrode. The standard buffer solutions for the colorimetric determinations were also checked by this method.

The results may be summarized briefly as follows. On the acid side of neutrality no change in the heliotropic response was seen until the pH approached the value of 6. At pH = 6 heliotropic swarming became less active and definite, and about 25 per cent of the larvae showed a negative response after a few minutes in the solution. At pH = 5.5 the negative response became general and the majority of larvae swam slowly toward the dark side of the vessel where most of them sank to the bottom. At pH = 5 few larvae showed any orientation; those which reacted to light were negative. Further increase of acidity quickly abolished all heliotropic response and swimming movements.

The reversal of heliotropism by mineral acids had previously been observed.⁹ It had also been shown that fatty acids are much more effective than mineral acids in causing reversal, but this effect is apparently independent of the H-ion concentration; *e.g.*, Kanda found that mere traces of valeric and caproic acids (0.00075 m. and 0.00006 m. respectively) in sea-water reversed heliotropism in 95 per cent of larvae within 15 minutes at 22° and within 5 minutes at 32°. ¹⁰

On the alkaline side normal positive swarming persisted until a pH of approximately 10 was reached. At pH = 10.5 heliotropism disappeared rapidly in most larvae, but a few remained positive for some time and a small proportion became negative. At pH = 11 all swimming movements ceased in a few seconds. The strong inorganic bases have thus only a slight effect in reversing heliotropism; ammonia is much more effective.¹¹ This difference between the strong and weak bases resembles that between mineral and fatty acids, and in both cases is probably to be correlated with a difference in the rate of penetration; the undissociated molecules probably also play an important part.

Arenicola larvae thus exhibit positive heliotropism in balanced media within a considerable range of H-ion concentration (pH = 6 to 10); on the acid side of this range reversal is readily produced, on the

¹⁰ S. Kanda: *loc. cit.*, p. 161.

¹¹ Kanda: *loc. cit.*, p. 163.

alkaline side less readily. The general physiological conditions of reversal are not understood. Mast has shown that the tonus of the musculature, whose varying state of contraction under different intensities of illumination determines the heliotropic response, is controlled by the stimulation of the eye spots.¹² Normally the musculature on the side of the more strongly illuminated eye is more contracted than that on the other side. Hence in a swimming larva placed with its long axis at an angle to the rays in a uniform light field the resulting body curvature (with concavity on the more illuminated side) soon steers the organism into a symmetrical position with head directed toward the source. Such a position is evidently a position of equilibrium, since it is the only position in which both eyes are equally illuminated and in which deviations are automatically corrected; evidently any deviation will tend to expose one eye more fully to the light and shade the other, thus causing a compensatory contraction of the musculature on the more illuminated side. Hence the organisms maintain their orientation in the field, and the continued ciliary movement carries them toward the source. A reversed position of equilibrium, *i.e.*, one with the head directed away from the source, would result if the musculature on the more illuminated side of the body were to undergo *relaxation* instead of contraction. Apparently the various reversing conditions alter the properties of the receptor-muscular arc in such a way that light stimulation now causes decrease instead of increase of muscular tone, *i.e.*, inhibition; but the precise conditions of this alteration are unknown. The transformation of a stimulating into an inhibiting condition (or *vice versa*) by changes in the composition of the medium is, however, not infrequent in organisms; it is also known that various inhibitions depend on the presence of salts in the medium or on the H-ion concentration.

The change from positive to negative heliotropism in *Arenicola* larvae seems to be associated with some kind of incipient cytolytic or injurious action; this is indicated by the general nature of the physical and chemical conditions under which reversal is produced. Conversely, positive heliotropism is an index of conditions under which the receptor-muscular arc functions normally; among these conditions the presence of a balanced proportion of salts and a normal H-ion range are shown by the above experiments to be essential. The specific importance of potassium for the persistence of the heliotropic reaction raises again the question of the rôle of potassium in cell-processes. Just where the

¹² S. O. Mast; *Light and the behavior of organisms*, New York, 1911, p. 172.

special influence of the potassium enters, in the path between the photoreceptor elements and the muscle fibers, cannot be said with certainty; but from the close analogies between the effects above described and the effects of potassium on muscular elements in general (especially on the vertebrate heart) it seems most probable that its chief action is exerted on the muscle cells and consists in maintaining the normal contractility and responsiveness of these elements. The fact that in heart muscle, containing an abundance of potassium in the cell-interior, the presence of a low and approximately constant proportion of K-salts in the external medium is necessary for normal activity¹³ seems further to indicate that some K-compound, *e.g.*, K-lipoid combination, is an indispensable constituent of the most external layer of the protoplasmic surface-film. This condition is probably general. We may conclude that in *Arenicola* larvae, with active cilia, positive heliotropism persists as long as the muscular elements preserve their normal properties and the conducting paths between eyes and musculature remain intact.

SUMMARY

1. The continuance of positive heliotropism in *Arenicola* larvae depends on the presence of a balanced proportion of salts (especially NaCl, CaCl₂ and KCl) in the external medium. An almost neutral or slightly alkaline reaction (pH = 6 to 10) is also a necessary condition.
2. Pure isotonic solutions of all salts instantly destroy the heliotropic response. The addition of CaCl₂ to isotonic NaCl solution enables heliotropism to continue for several minutes; MgCl₂ added to pure NaCl solution has little effect in prolonging heliotropism; when both MgCl₂ and CaCl₂ are added the prolongation of heliotropism is greater than that produced by either salt alone.
3. The addition of small quantities of KCl (3 to 5 volumes m/2 KCl in 100 volumes of solution) to balanced solutions of NaCl and CaCl₂ (and of NaCl, MgCl₂ and CaCl₂) enables heliotropism to continue for several hours. RbCl, but not CsCl, has a similar effect.
4. In the balanced isotonic solution of NaCl, MgCl₂, CaCl₂ and KCl slight acidity (pH = 6 to 5) renders the larvae negatively heliotropic; further increase of acidity (pH < 5) quickly destroys all heliotropic response. On the alkaline side positive heliotropism continues unchanged to pH 10; higher alkalinity (pH = 10.5 and greater) arrests the response but has only slight effect in causing reversal. H-ions are thus much more effective as reversing agents than OH-ions.

¹³ Cf. Clark: *loc. cit.*, p. 435; Daly and Clark: *Journ. Physiol.*, 1921, liv, 367.

INSULIN AND GLYCOLYSIS

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Received for publication May 14, 1923

The almost uniform rate at which the percentage of sugar declines in the blood of rabbits during the first half-hour or so following the injection of varying doses of insulin (1) would suggest the possibility that increased glycolysis in the blood itself might be the cause. At an early stage in our investigations, therefore, the rate of glycolysis in blood (defibrinated) removed from normal animals was compared with the same blood with insulin added, or in blood removed from an animal when the hypoglycemia due to injection of insulin had become pronounced. If insulin could be shown to alter the rate of glycolysis under either of these conditions, it would furnish us with a simple *in vitro* method for the pharmacological assay of this substance, and it would at the same time throw considerable light on the physiological mechanism of its action in producing hypoglycemia. In making these observations it was realized that the process of glycolysis in shed blood does not usually bear any relationship to the rate of sugar consumption in the intact animal (2) nor is it of any value in the diagnosis of diabetes. It was nevertheless thought of importance to investigate thoroughly the possibility that insulin accelerates blood glycolysis since no agent having so pronounced an effect on the percentage of blood sugar in the normal or diabetic animal had previously been available. The demonstration by Hepburn and Latchford (3) that sugar disappears more quickly from an artificial saline solution perfused through the excised heart when insulin is added to this fluid than otherwise, does not necessarily imply that absorption of sugar by the tissues is the only mechanism responsible for the decline of sugar in blood, because the same factors which cause the muscle cells of the heart to absorb the sugar might also act on the leucocytes of the blood.

The negative character of the results with blood alone led us step by step to investigate whether insulin could influence glycolysis in the other conditions in which glycolysis is believed to occur in animal fluids

and finally in conjunction with Dr. Andrew Hunter to see whether it had any effect on the hydrolysis of glucose by hydrogen peroxide or ferric chloride (4). Work on the last mentioned phase of the problem is not yet completed and the present paper deals exclusively with the effect of insulin on glycolysis in blood and in mixtures of blood or saline and Buchner extracts of muscle.

Since the experiments here reported were completed, Winter and Smith (5) have published a preliminary announcement in which they state that insulin activates a ferment present in extracts of liver so as to cause a conversion of α - β glucose into γ glucose, which is much more reactive and is presumably therefore more readily utilized by the organism. Grave doubts have however been cast by Hewitt (9) on the reliability of the methods used by Winter and Smith and we must await further confirmation of their work before accepting their results.

The particular problems dealt with in the present investigation are:

- a. Does insulin affect the rate of glycolysis in the defibrinated blood of the dog or rabbit, incubated outside the body under sterile conditions?
- b. Is the rate of glycolysis the same in the defibrinated blood of a normal animal as in that of the same animal some time after injection with insulin?
- c. Does insulin influence the rate of glycolysis in mixtures of muscle juice and blood or saline? (cf. Cohnheim, Hall (6).)
- d. Does insulin influence the rate of disappearance of sugar from sterile pus? (Levene and Meyer (7).)

METHODS. The blood was removed with precautions against bacterial contamination and was defibrinated by shaking in sterile flasks with glass beads. It was finally filtered through sterile gauze into small flasks which were placed in the incubator and shaken at frequent intervals. Samples of blood (1 cc.) were removed with sterile precautions at frequent intervals and the blood sugar determined by the Shaffer-Hartmann method. The observations were not usually prolonged over five hours so that accidental bacterial contamination could not become a complicating factor. In the observations of group c, rabbits were used, the muscle juice being prepared as follows: after stunning, the skin was stripped from the legs and a mass of the gluteal muscles removed and immediately chopped fine and ground in a mortar with quartz sand and infusorial earth. All this was done with sterile precautions. The plug was then enclosed in sterile sail cloth and the juice pressed out from it under 300 to 400 atmospheres pressure in the Buchner press. In one experiment, infusorial earth was omitted in case it might "adsorb" any active principle.

RESULTS. *A. Glycolysis in defibrinated blood. a. Rabbit blood.* Blood was collected from the carotid artery of a rabbit and after defibrinating by means of glass beads, it was divided into two equal portions, to one of which, A, 2 cc. of insulin were added and to the other, B, 2 cc. 0.9 per cent sodium chloride. Both portions were incubated at 37.0°C. and samples of 1 cc. removed at frequent intervals for analysis with the following results:

TIME	GLUCOSE (PER 100 CC. BLOOD)	
	A	B
<i>minutes</i>	<i>mgm.</i>	<i>mgm.</i>
0	149	143
30	124	114
60	113	90
90	96	92
120	92	77
150	73	56

Dog. In order to raise the concentration of sugar in the blood, a dog (9.5 kgm.) was injected subcutaneously with 30 cc. 1-10,000 adrenalin chloride and 40 minutes later 75 cc. of blood were withdrawn from the carotid artery and defibrinated in a sterile flask. The blood was divided into 2 portions, A and B, to the former of which 2 cc. of insulin were added and to the latter, 2 cc. of boiled insulin. The bloods were incubated at 38°C. and the following amounts of glucose were found at the intervals indicated:

TIME	GLUCOSE (PER 100 CC. BLOOD)	
	A	B
<i>minutes</i>	<i>mgm.</i>	<i>mgm.</i>
0	180	178
30	135	135
60	114	113
90	101	106
120	96	94
150	86	80
180	69	69
210	63	65

There is no significant difference in the rate of glycolysis up to 90 minutes at least. After this time the sugar disappeared somewhat more slowly from A (insulin blood) than from B.

There is very close correspondence between the rate of glycolysis in the two bloods, and the only fact that would make us hesitate in finally concluding from this experiment that insulin has no effect on glycolysis is that the control is not satisfactory, since it contained boiled insulin, and we have found, since performing this experiment, that insulin can withstand heating to a much greater extent than had been supposed to be the case at first, when less pure preparations (i.e., containing much protein) were used. Objection may also be taken to the experiment on the ground that epinephrin had been injected into the animal prior to taking the blood. There is no *a priori* reason to believe that this would alter the rate of glycolysis, and the close correspondence of these results with those in normal dog blood (2) support this.

The observation was repeated by Miss O'Brien, using blood removed from the heart of a dog, and adding to the control (A) in place of insulin an equal quantity of Ringer's solution of the same pH as the insulin. The following are the results:

TIME	GLUCOSE (PER 100 CC. BLOOD)	
	A	B
minutes	mgm.	mgm.
0	200	210
10	202	200
20	188	195
30	195	205
40	202	200
50	195	198
60	190	193
70	195	193
90	183	183
110	176	176
145	167	152

Glycolysis was unusually slow until after 70 minutes when it proceeded at approximately the same rate in both specimens.

These observations show that insulin does not accelerate glycolysis when added to defibrinated blood outside the body.

B. In this group glycolysis in blood removed from an animal after the injection of insulin is compared with that in normal blood. The procedure followed was to remove some blood, then inject insulin and an hour later remove more blood; the rate of glycolysis was then compared in the two samples of blood. Samples of blood were also removed at frequent

intervals from the animal after the injection of insulin so that the *in vivo* rate of glycolysis might be compared with that of the same blood *in vitro*.

Dog. 12.2 kgm. The pancreatic ducts of this animal had been tied some weeks previously, for another purpose. At 9:50 a.m. blood *A* was removed from the saphenous vein and defibrinated and placed in the incubator. Two cubic centimeters of insulin were then injected into the animal, and at 11:00 a.m. blood *B* was removed and placed in the incubator. The sugar was estimated in samples (of 1 cc. each) of blood removed from the flasks at regular intervals. Samples of blood were also removed from the animal at the same intervals as from the flasks, to show the rate of glycolysis in the animal itself, *C*. The following results were obtained:

	TIME						
	9:50	11:00	12:10	1:10	2:10	4:10	6:10
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
Glucose per 100 cc. { <i>A</i>	94	83	67		28	12	
{ <i>B</i>		74	46	20	20	20	
{ <i>C</i>			48	43	56	69	95

It is evident from *C* that insulin of high potency was used, the maximum degree of hypoglycemia being reached in 3 $\frac{1}{4}$ hours after injecting the insulin.

Comparison between *A* and *B* shows after one hour that sugar disappeared more rapidly from the blood within the body of the injected animal than from the incubated (normal) blood of the same animal. This is not to be wondered at, because the temperature of the shed blood may have temporarily been below that of the body. A similar difference is observed during the next hour, when both the normal blood and that removed from the insulin-injected animal were compared. Subsequently, however, the two bloods (*A* and *B*) contained the same amounts of glucose. This experiment was repeated in the same order on another dog with the following results:

	TIME						
	10:50	12:00	1:00	2:00	3:00	4:00	5:00
	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Per cent glucose { <i>A</i>	0.094	0.085	0.086	0.032	0.020	Trace	
{ <i>B</i>		0.057	0.073	0.028	0.016	Trace	
{ <i>C</i>			0.046	0.049	0.051	0.058	0.60

As seen from *C*, massive doses of insulin (given in two doses at 10:50 and 11:40) were also injected into this animal but the rate of glycolysis was practically the same in *A* (normal blood) as in *B* (blood removed after insulin), except for the sample removed at 12:00 which is decidedly below that of *A*.

A similar experiment performed on the rabbit gave the following results:

September 11th. Rabbit (weight 2030 grams). Given 4 cc. insulin at 12:05 p.m. and bled at 1:45 p.m. after stunning. The blood was run through sterile gauze into sterile flasks containing glass beads. After defibrination 0.15 cc. 10 per cent dextrose was added to 10 cc. of blood, and the mixture incubated at 34°C. (a).

Rabbit (weight 2300 grams). Bled in a similar way at 1:35, after stunning. To 20 cc. of the defibrinated blood was added 0.20 cc. 10 per cent dextrose solution, and the mixture divided into two equal parts of 10 cc. each. To the first of these (b) 1 cc. of insulin was added; to the second (c) 1 cc. insulin which had been boiled for 3 minutes; the flasks were kept in the incubator for about 4½ hours, and samples of the contents removed for analysis at the times shown.

BLOOD OF INJECTED RABBIT (a)		BLOOD OF NORMAL RABBIT			
		With unboiled insulin (b)		With boiled insulin* (c)	
Time	Glucose per 100 cc.	Time	Glucose per 100 cc.	Time	Glucose per 100 cc.
<i>p. m.</i>	<i>mgm.</i>	<i>p. m.</i>	<i>mgm.</i>	<i>p. m.</i>	<i>mgm.</i>
1.55	234	2.00	264	2.03	272
2.25	230	2.30	257	2.33	221
2.55	212	3.00	242	3.03	243
3.25	197	3.30	231	3.33	234
3.55	184	4.00	187	4.03	229
4.25	183	4.30	180	4.33	205
Percentile glycolysis in 150 minutes.....	21.8		30.8		24.2

* We have recently shown that the purer preparations of insulin now obtainable withstand actual boiling for at least one hour, without perceptible change in potency.

Comparing b and c, there was no difference in the rate of glycolysis during the first 90 minutes of incubation, although it was slightly quicker in the blood containing unboiled insulin during the next 60 minutes. The blood of the rabbit previously injected with insulin (a) underwent glycolysis somewhat more slowly than either of the others.

Although evidence furnished by these experiments is conclusive in showing that insulin does not affect the rate of glycolysis either in shed blood or in blood removed from the animal some time (100 minutes) after injecting insulin, there remains the possibility that it might be accelerated in blood removed within a few minutes of injecting the insulin. The above observation was therefore repeated by comparing the rate of glycolysis in the blood of a normal rabbit with that of blood removed from another rabbit in about 15 minutes after injecting a massive dose of insulin, and glycolysis was found to be decidedly more rapid in the latter case. The experiment could not, however, be considered as conclusive because of a possible variability in the rate of glycolysis in normal blood. As a matter of fact, it was found that this is far from being the same in the blood of different normal rabbits. The observation was therefore repeated by first of all removing some blood (3 to 4 cc.) from the ear vein, then injecting insulin and removing more blood, about 10 minutes later. Both bloods after defibrination were then placed in a water bath at 38°C. and samples of 0.1 cc. removed at regular intervals for determination of sugar by the Hagedorn-Jensen method (8), controls being made at the beginning and end of the incubation period by that of Shaffer and Hartmann. About 4 cc. of blood were removed from the ear vein of a large rabbit and defibrinated in a flask which was then placed in a water bath at body temperature (A). A large dose of insulin was injected and another 3 to 4 cc. of blood removed in 15 minutes and treated like the control (B). Samples of 0.1 cc. of blood were removed from both flasks at 5-minute intervals and the sugar measured by the Hagedorn-Jensen micro method with the following results:

TIME AFTER START	GLUCOSE PER 100 CC. BLOOD	
	A (normal)	B (insulin)
<i>minutes</i>	<i>mgm.</i>	<i>mgm.</i>
0	171	156
5	192	152
13	186	152
22	166	153
26	159	
29		148
32	166	
40		148
46	163	148

Although somewhat irregular the results show no difference in the rate of glycolysis.

C. In light of the results obtained by Cohnheim and others already referred to, several observations were made to see whether insulin might accelerate the rate of disappearance of glucose from sterile mixtures of blood or saline and muscle or Buchner extract of muscle. This was done partly by adding insulin to the mixtures of blood and muscle prepared from normal animals and partly by using blood and muscle removed from animals under the influence of insulin.

September 13th. *Rabbit* given 5 cc. insulin at 9:32 a.m. and bled at 11:15 a.m., the defibrinated blood being used in mixtures a and b. The blood of a normal rabbit killed at 11:30 was used for mixtures c and d. The phosphate mixture contained 25 cc. M/5 KH_2PO_4 + 100 cc. M/5 Na_2HPO_4 . The following mixtures were made, the muscle being removed with sterile precautions.

- (a) 20 cc. blood; 6 cc. phosphate; 0.8 cc. dextrose; (10 per cent).
- (b) 10 cc. blood; 3 cc. phosphate; 0.8 cc. dextrose; 5 gms. chopped muscle.
- (c) 10 cc. blood; 3 cc. phosphate; 0.8 cc. dextrose.
- (d) 10 cc. blood; 3 cc. phosphate; 0.8 cc. dextrose; 5 gms. chopped muscle.

These mixtures were incubated for about 4 hours, and at periods samples were removed for determination of sugar.

Analyses

TIME	GLUCOSE PER 100 CC.			
	Injected rabbit		Normal rabbit	
	(a)	(b)	(c)	(d)
	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
11:45 a.m.	211	192	216	227
12:15 p.m.	200	183	205	200
1:15 p.m.	200	186	190	173
2:30 p.m.	188	187	164	145
4:00 p.m.	(185)	(207)	135	125
Percentile glycolysis in 165 minutes.....	10.9	12.6	24	36

Comparison of a and b shows that the presence of muscle does not significantly influence the rate of glycolysis in the blood of insulin-injected animals although, as comparison of c and d shows, it appears to do so slightly in the blood of normal rabbits. The rate of glycolysis in the blood and in the mixture of blood and muscle of the insulin-injected animal (a and b) was considerably less than that in the normal animal (c and d).

September 15th. Normal rabbit used. The pipettes were boiled instead of being placed in the autoclave. Muscle juice was obtained by the Buchner press at 300 atmospheres (mixed with sand and infusorial earth).

(a) 10 cc. defibrinated blood, 2 drops 10 per cent dextrose; 3 cc. phosphate mixture (referred to above); 3 to 4 cc. muscle juice.

(b) 10 cc. blood; 2 drops dextrose; 3 cc. phosphate; 3 to 4 cc. muscle juice; 1 cc. skate insulin.

(c) 2 drops dextrose; 3 cc. phosphate; 3 to 4 cc. muscle juice.

(d) Same as (c) plus 1 cc. skate insulin.

They were analyzed immediately after mixing and then incubated.

Analyses

TIME	GLUCOSE PER 100 CC.			
	(a)	(b)	(c)	(d)
	mgm.	mgm.	mgm.	mgm.
2:20 p.m.			264	246
2:50 p.m.	158	152		
3:20 p.m.	136	158	248	226
3:50 p.m.	123	126		
4:20 p.m.	140	133	259	255
4:50 p.m.	135	152		
5:20 p.m.	100	143	251	241
5:50 p.m.	116	136		
6:20 p.m.	82	124		235
Percentile glycolysis in 180 minutes.....	26.5	10.5		

Although the results are less regular than usual, glycolysis is seen to be decidedly retarded by the presence of insulin and there is no evidence of glycolysis having occurred in the muscle juice alone, either with or without insulin.

The following experiment was done to see if muscle juice would accelerate blood glycolysis when both it and the blood were removed from an insulin-injected animal.

September 19th. 9:53 a.m. Rabbit given 4 cc. insulin (two rabbit doses). Killed, bled, etc., at 11:23 a.m., as before. The muscle which was obtained under sterile precautions was ground with sterile sand and infusorial earth, wrapped in sterile sail cloth, and pressed in a Buchner press at 300 atmospheres.

(a) 10 cc. defibrinated blood; 5 cc. of phosphate mixture; 5 cc. normal saline; 3 drops 10 per cent dextrose.

(b) 10 cc. defibrinated blood; 5 cc. muscle juice; 5 cc. phosphate; 3 drops 10 per cent dextrose.

(c) 5 cc. muscle juice; 5 cc. phosphate; 10 cc. normal saline; 3 drops 10 per cent dextrose.

The mixtures were immediately analyzed and then incubated at 38°C.

Analyses

TIME	GLUCOSE PER 100 CC.		
	(a)	(b)	(c)
	<i>m./m.</i>	<i>m./m.</i>	<i>m./m.</i>
12:15 p.m.	118	209	173
1:45 p.m.	96	212	165
2:45 p.m.	(103)	217	168
4:45 p.m.	87	218	189
5:45 p.m.	89	196	192
7:20 p.m.	67	185	177
8:20 p.m.	56	156	156
9:20 p.m.	56	156	130
10:20 p.m.	34	62	?

Glycolysis was decidedly more rapid in blood alone (a) than in blood plus muscle juice (b) or in muscle juice alone (c). Up to 5 hours after the start of incubation there was practically no glycolysis either in blood plus muscle juice or in muscle juice alone, and after this time the glycolysis which did occur in these fluids may have been due to bacteria. The unexpected results of this experiment led us to repeat Cohnheim's observations according to his directions with the results described in the next experiment.

September 25th. Rabbit 1 was given 4 cc. insulin at 10:30 a.m. and killed at 12 noon. The blood sugar in the defibrinated blood was 63 mgm. per 100 cc. Normal rabbit 2 was killed at 10:45 a.m., the blood obtained and defibrinated, and muscle juice prepared by Buchner's process. These were kept in the ice-chest until ready. The pancreas of this rabbit (2) was thrown into boiling water, evaporated almost to dryness; alcohol was added, filtered off, evaporated to dryness and taken up in distilled water (Cohnheim). The following mixtures were made:

(a) 10 cc. blood from rabbit 2; 4 cc. muscle juice; 4 cc. usual phosphate mixture; 2 drops 10 per cent dextrose solution.

(b) 10 cc. blood from rabbit 2; 4 cc. muscle juice; 4 cc. phosphate 2 cc. insulin; 2 drops 10 per cent dextrose solution.

(c) 10 cc. from rabbit 2; 4 cc. muscle juice; 4 cc. phosphate; 2 drops 10 per cent dextrose solution; pancreas preparation described above.

(d) 10 cc. blood from rabbit 1; 2 cc. muscle juice; 2 cc. phosphate; 2 drops 10 per cent dextrose solution.

(e) 10 cc. saline; 2 cc. muscle juice; 2 cc. phosphate; 2 drops 10 per cent dextrose solution.

Analyses

TIME	GLUCOSE PER 100 CC.				
	(a)	(b)	(c)	(d)	(e)
	mgm.	mgm.	mgm.	mgm.	mgm.
12:35 p.m.	180	185	173	129	85
1:35 p.m.	140	182	157	111	82
2:35 p.m.	160	182	157	113	75
3:35 p.m.	137	167	135	99	91
4:35 p.m.	137	162	137	77	80
5:35 p.m.	119	155	119	62	83
Percentile glycolysis after 3 hours.....	23.8	9.1	22.0	25.2	

The rate of glycolysis was the same in blood plus muscle juice (a) and blood plus muscle juice plus Cohnheim's extract (c). It was, however, decidedly slower when insulin was added (b). During the first 3 hours of incubation glycolysis was practically the same in mixtures of blood and muscle juice (a) as in the blood of the insulin-injected animal and muscle juice (d). It should be observed however that 90 minutes elapsed between the injection of insulin and the removal of blood.

This experiment was repeated with similar results, that is, insulin decidedly retarded the glycolysis occurring in mixtures of blood and muscle juice, whereas Cohnheim's extract had no influence.

October 2nd.

(a) 10 cc. blood; 4 cc. phosphate mixture; 4 cc. muscle juice; 1 cc. saline; 2 drops 10 per cent dextrose solution.

(b) 10 cc. blood; 4 cc. muscle juice; 4 cc. phosphate; 1 cc. insulin; 2 drops 10 per cent dextrose solution.

(c) 10 cc. blood; 4 cc. muscle juice; 4 cc. phosphate mixture; 1 cc. pancreas preparation described above; 2 drops 10 per cent dextrose solution.

Analyses

TIME	GLUCOSE PER 100 CC.		
	No insulin (a)	Insulin (b)	Pancreas preparation (c)
	mgm.	mgm.	mgm.
12:30 p.m.	192	160	192
1:30 p.m.	163	158	176
2:30 p.m.	156	164	158
3:30 p.m.	137	146	138
4:30 p.m.	131	140	134
5:30 p.m.	115	137	123
Percentile glycolysis after 4 hours.....	31	12.5	30

The following experiment shows that insulin does not influence the rate of glycolysis in Buchner extract of muscle *plus* phosphate mixture.

October 9th. This experiment was a repetition of the preceding one.

(a) 5 cc. muscle juice (Buchner process); 2.5 cc. phosphate; 2 drops 10 per cent dextrose solution.

(b) 5 cc. muscle juice; 2.5 cc. phosphate; 2 drops 10 per cent dextrose solution; 2 cc. insulin.

TIME	GLUCOSE PER 100 CC.	
	(A) No insulin	(B) Insulin
	mgm.	mgm.
10 a.m.	269	257
11 a.m.	277	225
12 m.	267	242
1 p.m.	250	254
3 p.m.	235	261

The most constant result obtained in these observations is that insulin actually retards the rate of glycolysis both in blood and in mixtures of blood and muscle. It has, therefore, exactly the opposite influence from that which was being looked for. This result may be due to the fact that the solutions of insulin are sometimes decidedly acid in reaction (pH below 4) although with the relatively small amounts of this fluid added and the presence in many experiments of buffer substances, this does not seem likely to be the explanation for the result. The skate insulin used in the experiment of September 15th, for example, was nearly neutral. We are at a loss to explain the result.

D. In view of Levene and Meyer's (7) experiments showing that sterile pus incubated outside the body in the presence of phosphates is capable of causing glucose to disappear, partly by conversion into lactic acid, experiments were undertaken to see whether insulin might accelerate this process.

January 17th. Pus resulting from the injection of turpentine, several days previously into the pleural cavity of a dog, was removed with aseptic precautions and mixed in two sterile flasks as follows:

(a) 25 cc. pus; 15 cc. M/5 phosphate of pH 7.3 to 7.4; 5 cc. 2 per cent glucose; 1 cc. insulin; 4 cc. Ringer's solution.

(b) 25 cc. pus; 15 cc. phosphate; 5 cc. 2 per cent glucose; 5 cc. Ringer's. A third unsterile flask was prepared containing 15 cc. pus; 9 cc. phosphate; 3 cc. glucose; 3 cc. Ringer's. The glycogen was estimated in this, and found to be 0.005 per cent. The pus was removed at 11:30 a.m.

TIME	GLUCOSE PER 100 CC.	
	(A)	(B)
	mgm.	mgm.
11.40	175	170
12.10	170	151
12.40	170	137
1.40	137	138
2.40	137	117
3.40	127	112
4.40	117	102
5.40	82	80
9.40	20	28
10.40	24	18
	87.4 per cent	86.8 per cent
After hydrolysis with HCl for 10 minutes		
	45	24
Glycogen.....	0.003 per cent	0.001 per cent

It is clear that insulin has no effect on the rate at which sugar disappears from the sterile pus.

The above experiment differs in detail from those of Levene and Meyer in that the pus cells were not washed with isotonic saline and the period of incubation lasted only 11, instead of 36 hours. Taking the average of the amount of sugar left after 10 and 11 hours, the percentile glycolysis was 87.4 with insulin, and 86.8 without, indicating quite clearly that insulin had no significant influence on the process. For the first 4 hours glycolysis proceeded in the insulin preparation somewhat more slowly than in the control flask. This agrees with the results obtained with blood and is difficult to explain. It will be observed that glycolysis was decidedly more rapid in our preparations than in those used by Levene and Meyer, but a part of this greater speed, viz., that occurring beyond the fourth hour, may have been due to bacterial growth, even though sterile precautions were taken. At this period, however, over 30 per cent of the original sugar had disappeared, whereas in Levene and Meyer's experiment only about 10 per cent disappeared in 36 hours. These discrepancies are probably dependent upon the differences in the concentration of glucose in the mixtures, this being much greater in Levene and Meyer's experiments than in ours. The disappearance of sugar in mixtures containing leucocytes, instead of being due to destruction of glucose, as was actually

demonstrated to occur by Levene and Meyer, might also depend on a polymerization of glucose into glycogen within the leucocytes. To ascertain whether this occurs, the glycogen was determined by Pflüger's method, before and after the incubation. Minute traces only were found, and if anything, there was less after the 10 hours' incubation.

Experiments were also undertaken to see if insulin could influence the rate at which glucose is fermented by yeast. For this purpose baker's yeast (Fleischmann's) was ground up with a 1 per cent solution of glucose, and equal quantities of the resulting suspension placed in a series of fermentation tubes. Two of these served as controls and to the others were added varying quantities of insulin. After standing at room temperature for varying periods no difference could be detected in the rate of gas formation in the various tubes.

DISCUSSION. Although glycolysis was not exactly uniform in the various observations herein recorded—probably because the flasks were not constantly agitated during incubation—there can be little doubt that insulin does not affect it, at least in the defibrinated blood of the rabbit or dog. The rapidly developing hypoglycemia which occurs in these animals following the injection of insulin would therefore appear to be due to an extravascular or intracellular process. In other words, when insulin is injected into an animal it must pass from the blood through the capillaries into the tissue cells and cause therein a rapid disappearance of glucose—a vacuum for this substance, as it were, with the result that glucose also is removed from the blood at a rate which is greater than can be made good by a new production of glucose from the glycogen reserves. The possible nature of the process by which the cells cause the glucose to disappear will be discussed in future papers.

Winter and Smith¹ have announced that they have found that the insulin acts by activating a ferment, derived partly from the liver, which then accelerates the conversion of $\alpha\beta$ glucose into γ glucose which is apparently much more reactive.

If γ glucose were formed in any considerable quantity in the blood, following insulin injections, it seems reasonable to expect that there would be some difference in the rate of glycolysis in blood removed after injecting insulin and in the normal blood of the same animal. This is not the case. It may of course be that the activation of glucose does not take place in the blood itself but only in the tissue cells and some support

¹ Hewitt (9) has recently given very strong reasons for questioning the reliability of the methods used by Winter and Smith.

is lent to this view by the fact that insulin markedly increases the rate of sugar consumption by the excised heart perfused with Ringer's solution.

Our inability to demonstrate that insulin affects the rate of glycolysis in mixtures of blood, or saline, and chopped muscle or muscle juice, shows that the more rapid disappearance of glucose in the tissues as a result of insulin injection is dependent upon the integrity of the cell. Restated, then, the problem to be solved is whether insulin acts in the blood—possibly in conjunction with some circulating co-enzyme—to form, an active (γ ?) form of glucose or a compound of glucose which then passes into the tissue cells (including those of the liver) or whether it passes into the tissue cells before it causes these changes.

CONCLUSIONS

1. The addition of insulin to the defibrinated blood of the rabbit and dog does not affect the rate of glycolysis in vitro.

2. This is also the same in normal blood as in blood removed from these animals after injecting insulin, both when the blood is removed within a few minutes after injecting the insulin and when it is removed after the hypoglycemia has become pronounced.

3. Addition of insulin to mixtures containing blood or saline and Buchner extract of muscle does not alter the rate of glycolysis.

4. Glycolysis is also the same in similar mixtures made from normal animals as in those made from animals recently injected with insulin.

5. Insulin does not influence the rate of glycolysis in a suspension of leucocytes.

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PHYSIOLOGICAL EVIDENCE OF THE EXISTENCE OF A NON-VISUAL AFFERENT MECHANISM IN THE EYE

I. VASOMOTOR RESPONSES TO INTENSE LIGHT

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Received for publication May 15, 1923

This investigation was suggested by the observation of a case in which an individual was so affected by bright sunlight as to suffer pronounced prostration characterized by cardiac disturbances followed by muscular weakness. This was not commonly accompanied by headache such as comes from ordinary ciliary fatigue, nor was the effect delayed as in such cases but on the contrary sometimes came on within ten minutes.

At the time the observation was first made the patient was suffering from a degree of hyperthyroidism sufficient to give a metabolic index of 66+ as a result of which there was increased sensitiveness to all external stimuli. The fact of hypersensitiveness, the early onset, the absence of headache, were suggestive of involvement of some mechanism whose action is not ordinarily recognized in the usual reactions responsive to bright light.

This possibility received some support in the observations of Arey (1) that in the optic nerve of the catfish non-optical elements were observed. Also, Tozer and Sherrington (2) have demonstrated that in the monkey the third, fourth and sixth nerves are afferent-efferent, the afferent elements being proprioceptive. And quite recently Nicholson (3) has observed ganglion cells on the third and sixth nerves of a full term human fetus.

In the full term human foetus, nerve cells occur on the sixth and third nerves within the orbit. A small ganglion is situated on the periphery of the sixth nerve at its entrance into the orbital cavity through the superior orbital fissure. On the third nerve several cell groups occur. There are two separate groups on the branch supplying the inferior oblique muscle. One or two nerve cells occur on the branch supplying the internal rectus muscle; there are none on the fourth nerve.

She has not determined whether these are proprioceptive or sympathetic. These observations, while not closely correlated nor extensive, indicate that there may be present in the ocular structure some mechanism not ordinarily recognized but nevertheless capable of exercising considerable influence on bodily reactions.

It seems improbable, however, that the vaso-motor reaction following strong illumination of the retina is due to stimulation of sensory elements in the oculo-motor nerves.

In experimental procedures dogs were used exclusively. The left eye was atropinized to complete dilatation before anesthesia. Ether was used as an anesthetic in four cases. In all other experiments chlorotone was employed in 10 per cent solution in cottonseed oil, injected intraperitoneally; average dose 0.35 gram per kilogram of body weight. Anesthesia was complete in five to ten minutes.

The lids of the atropinized eye were held open with a wire speculum. A 500 watt baloptican was placed about six feet away and so adjusted that the axis of the beam therefrom coincided with the visual axis. The speculum was applied about two minutes before the light was turned on. The eye was not manipulated in any way while the light was on. Blood pressure was recorded from the carotid artery in the usual way. Opening the eyelids did not affect blood pressure.

Results: Fifty-one trials were made on twenty-seven dogs, in all of which blood pressure was recorded. In thirty-five instances, heart-rate was also recorded. The results are shown in table I.

Blood pressure was decreased in 31 or 60.7 per cent of cases and in 16 of these or 51 per cent the fall was accompanied by a corresponding decrease in heart rate, this being the most predominant effect on the heart. While there were some few instances of increase in heart rate and blood pressure the two factors were not increased simultaneously in any single case.

In 21 of the 31 cases of decreased blood pressure there was partial or complete recovery after the light was turned off. There was never continued fall after the stimulus was discontinued. As was to be expected, there was usually a rather long latent period. On the other hand, in comparison with the observed reactions already mentioned in the human, the experimental effects came on quickly, considering the depth of anesthesia. It was noticeable that effects were always more pronounced in the earlier stages of the experiment, whether due to less profound anesthesia or to adaptation in later stages, was not determined, though the latter explanation seems most probable.

The following briefs will give a fair notion of the course of events in most experiments:

Exper. 20; dog, 7.4 kilos; 0.3 gram per kilo chloretone; blood pressure 77 mm.; heart rate 178; light turned on 3.4 minutes; average blood pressure during 2nd minute, 51 mm.; heart rate 172; during 3rd minute, b.p. 12; h.r. 120; end of 5 minutes after light turned off, b.p. 36, h.r. 142; after 25 minutes, b.p. 41, h.r. 146.

Exper. 21; blood pressure 50, heart rate 130; light 2 minutes, b.p. 41, h.r. 124; light off 1 minute, b.p. 45; h.r. 132.

Exper. 41; blood pressure 110, heart rate 196; light 4 minutes, b.p. 85, h.r. 192; light off, no immediate change; 1 hour 15 minutes after b.p. 90, h.r. 192.

TABLE 1

BLOOD-PRESSURE	HEART-RATE			
	Number of times increased	Number of times decreased	Not affected	Not recorded
Number of times increased (8 = 15.6 per cent).....			1	7
Number of times decreased (31 = 60.7 per cent).....	7	16	2	6
Number of times not affected (12 = 23.5 per cent).....	1	1	7	3
Totals.....	8	17	10	16

The graph shown in figure 1 (exper. 20) is typical of the more pronounced effects, showing an extensive and sudden fall in blood pressure and decrease in heart rate with partial recovery at the end of 25 minutes after the stimulation was discontinued.

In most instances recovery, complete or partial, did not occur until after the stimulus was discontinued. But in a few instances there was only a very slight fall in blood pressure with complete recovery while the stimulus was still being applied, showing that, whatever the depressing mechanism, a minimal stimulus is not long effective.

The predominant effect, then, of stimulation of the atropinized eye with intense light seems to be a fall in blood pressure with an accompanying decrease in heart rate with a fair degree of recovery of both factors in the majority of cases.

The question arises as to how these effects are brought about. The first possibility that is suggested is that the reaction is due to pain stimuli resulting from the intense light. Stimulation with a strong induced current of various structures—conjunctive, ciliary region, etc.—always elicited a slight depression.

The question of the action of the chloretone must also be considered. Sollman (4) states that this drug lowers blood pressure in a dog very slowly. Rowe (5) states that pulse rate is not affected and blood pressure remains constant. Our own results show a slight initial fall of blood pressure with constant level thereafter. No experimental manipulations were attempted until this level had been reached.

Nevertheless four controls were run, in two of which blood pressure and heart rate remained unchanged for one and one-half hours, while in the other two both pressure and heart rate rose slightly in the same

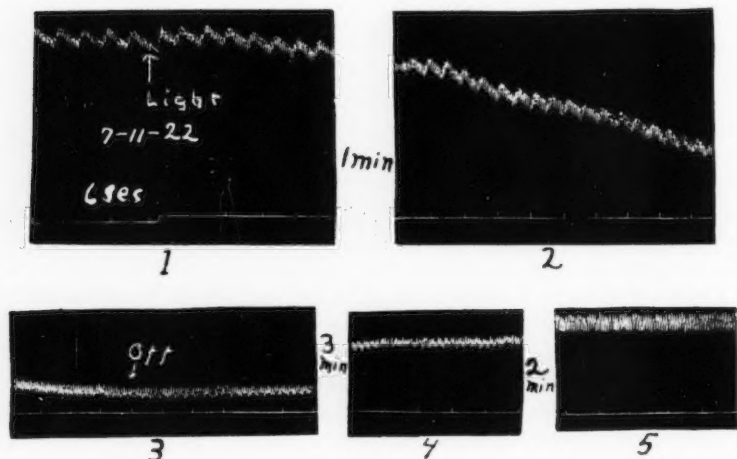


Fig. 1. Exper. 20. Effect on blood pressure of intense light. One minute, interval 1-2; $\frac{1}{2}$ minute, 2-3; 3 minutes, 3-4; 2 minutes, 4-5. No change in 10 minutes after 5.

period. In some other instances a very slight fall was observed over periods of one-half hour but nothing simulating in any way the results observed in relation to light. Furthermore, all animals in which ether was used showed positive responses to light amounting to an average of 10 per cent lowering of blood pressure.

The sixth nerve was now isolated within the orbit and stimulated with strong tetanizing current. Typical responses were shown in other tracings as a slight but definite fall in blood pressure outlasting the stimulus several seconds. It was not found possible to stimulate this nerve sufficiently to produce any more pronounced results than

those shown. No attempt has yet been made to determine whether the response results from a stimulus due to light rays, heat rays or ultra-violet rays.

CONCLUSIONS

It appears then that there must be some part of the ocular mechanism that is responsive to intense light and that the responses affect the vasomotor mechanism very extensively; the predominant effect is one of depression. The means by which this is brought about are difficult to trace and a satisfactory explanation has not been produced so far.

Thanks are due Dr. G. E. Coghill and Miss Helen Nicholson of the Department of Anatomy for permission to quote in advance from the latter's unpublished thesis, and to Dr. O. O. Stoland for helpful criticism.

Expenses of this investigation were defrayed by a grant from the Graduate Research Committee, University of Kansas.

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AFFERENT RELATIONS OF THE SKIN AND VISCERA TO THE PUPIL DILATOR MECHANISM

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Received for publication May 16, 1923

Method. In previous papers the author discussed the occurrence of paradoxical (1), (2) and pseudo-paradoxical (3) pupil dilatation following lesions of the somatic afferent paths. As the present contribution deals with the occurrence of these phenomena after skin and visceral lesions, the reader is referred to the aforesaid papers for the general method of study. A knowledge of the mechanism and time relations of these two sets of phenomena is a pre-requisite for their differentiation. In the present studies the animals were deeply anesthetized with ether and the organs injured mechanically by needle puncture or by crushing with an artery clamp. Wherever possible acupuncture was done from the outside, i.e., through the abdominal or thoracic wall, without opening the chest or abdominal cavity, care being taken to balance the puncture wound of the thoracic or abdominal wall by a wound of similar extent at the corresponding point on the opposite side. Wherever an organ had to be reached by incision this was made in the median line. The right ventricle of the heart was reached (in an average sized adult cat) in the median line 6.5 cm. cephalad of the tip of the xiphoid cartilage, the needle being plunged in an antero-posterior direction slightly caudalward and to the left. The left ventricle was reached from the same point (a) by plunging the needle still more caudalward and to the left or (b) through the right ventricle. The auricles and aortic arch were reached in the middle line at a point 8.0 cm. cephalad of the tip of the xiphoid cartilage. All lesions were verified at autopsy.

Experimental facts. Lesions in the following situations were found to be associated with pseudo- and true paradoxical phenomena in the homolateral eye: (a) Skin of side of face and head. (b) Skin of one side of thorax above level of umbilicus. (c) Kidney cortex, secreting system (5 animals acupuncture) figure 1. (d) Kidney as a whole

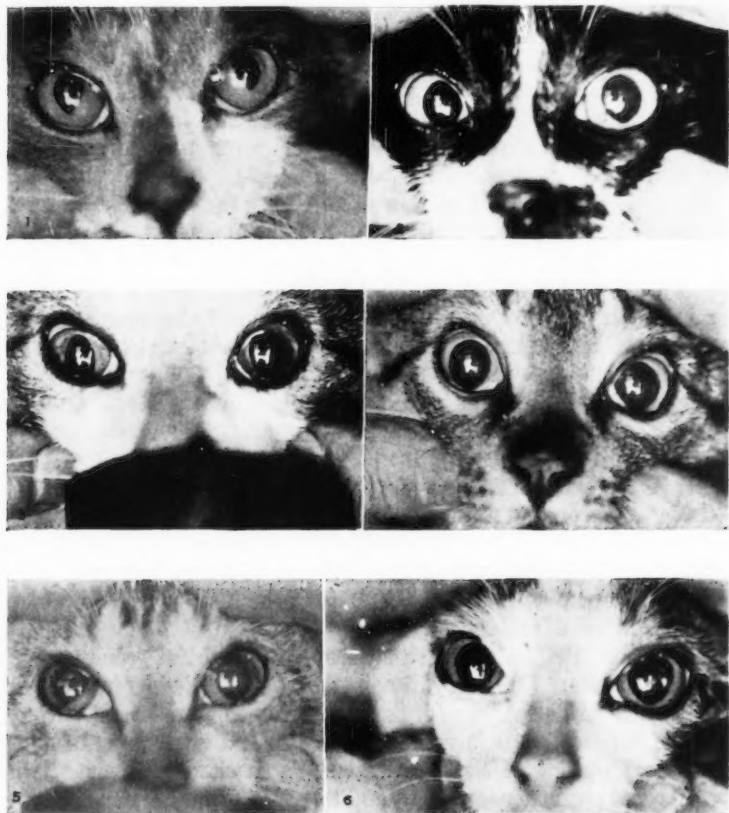


Fig. 1. True paradoxical dilatation of right pupil on 7th day after cortical lesion of right kidney.

Fig. 2. True paradoxical dilatation of left pupil on 15th day after crushing upper portion of right ureter.

Fig. 3. True paradoxical dilatation of left pupil on 8th day after acupuncture of left testis.

Fig. 4. True paradoxical dilatation of left pupil on 8th day after crushing right epididymis.

Fig. 5. True paradoxical dilatation of left pupil on 15th day after acupuncture of left ovary.

Fig. 6. True paradoxical dilatation of left pupil on 15th day after acupuncture of right side of uterus.

(removal, 7 animals). (e) Anterior (left) wall of stomach (3 animals, acupuncture). (f) Posterior (right) wall of stomach (2 animals, crushing). (g) One lung (3 animals, acupuncture). (h) Right ventricle of heart (acupuncture, 7 animals). (i) Left ventricle of heart (acupuncture 3 animals). (j) Arch of aorta, left ventricular system (acupuncture 3 animals). (k) Spleen (acupuncture, 2 animals) (1). Right side of duodenum (crushed, 2 animals). (m) Thyroid gland, crushed. (n) Adrenal gland, acupuncture. (o) Testicle, secreting system (acupuncture in 2, crushing in 3 animals) figure 3. (p) Testicle as a whole (crushed 4 animals). (q) Ovary, secreting system, (acupuncture one animal, crushing, one animal,) figure 5.

Lesions in the following situations were found to be associated with pseudo- and true paradoxical phenomena in the contralateral eye: (a) Kidney, duct system, i.e., pelvis, ureter, (acupuncture, 2 animals; crushing, 2 animals) figure 2. (b) Testicle, duct system, i.e., epididymis, etc. (acupuncture, 2 animals; crushing, 2 animals) figure 4. (c) Uterus and vagina, unilateral injury (acupuncture, 2 animals) figure 6. (d) Uterine (Fallopian) tube, (crushing, 2 animals). (e) Bladder wall and urethra (unilateral acupuncture, 2 animals). (f) Appendix caeci, either side in relation to mesentery (acupuncture, 3 animals). (g) Ileum, 6 inches cephalad of ileo-caecal valve, either side in relation to mesentery. (h) Skin of the hind quarter (anywhere caudad of level of umbilicus).

Lesions of the following situations gave doubtful, conflicting or negative results: (a) Liver in 5 animals. (b) Gall bladder in 2 animals. (c) Pancreas in 2 animals. (d) Parietal peritoneum, unilateral mechanical injury (scratching) in subdiaphragmatic and lower left abdominal regions. Crushing of the whole appendix caeci was associated with paradoxical phenomena, pseudo- and true, in the contralateral eye.

In a further effort to trace the mechanism by which these effects are accomplished, both vagi were cut in five dogs already found to be responsive. In no case was the typical response modified.

DISCUSSION AND SUMMARY. The results after skin lesions corroborate those already found after unilateral injury of the sciatic nerve (1), brachial plexus or cervical nerves (2) and 5th cranial nerve (3). Roughly, it seems that skin regions whose afferent nerves enter the spinal cord for the most part below the level of about the 10th thoracic segment have preponderate functional relations with the dilator mechanism of the contralateral eye, whereas skin regions whose afferent nerves enter the cord for the most part at levels higher

Protocol. Cat 24. Male, adult.

DAY	TIME	REMARKS	PUPILS		MEMBRANES	
			Right	Left	Right	Left
	<i>a. m.</i>		<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
2nd	8:18	March 24, 1923. Punctured left testis through the skin and made an equalizing skin lesion over the right testis				
	8:20	Pseudo-paradoxical dilatation in left pupil	6.0	7.0	1.0	1.0
	10:15	Pseudo-paradoxical dilatation in left pupil	2.5	3.0	2.0	2.0
	10:18	Ether				
	10:30	Under ether; wink reflex absent; pseudo-paradoxical dilatation absent in left eye	1.5	1.5	4.5	5.0
8th	10:30½	Adrenalin m ii; true paradoxical phenomena absent in left eye	5.0	5.0	2.0	2.0
	9:20	Pseudo-paradoxical dilatation absent in left eye	6.0	5.0	1.5	2.0
	9:24	Ether				
	9:38	Wink reflex present; trace of true paradoxical phenomena in left eye	3.0	3.5	3.0	3.0
	9:38½	Adrenalin m ii; true paradoxical phenomena in left eye	11.0	13.0	0.0	0.0
12th	9:39½	In constriction after adrenalin. Figure 3	7.0	9.0	2.5	2.0
	7:55	Pseudo-paradoxical dilatation in left pupil	2.5	3.0	1.5	1.5
	7:56	Ether				
	8:02	Wink reflex absent; true paradoxical phenomena in left eye	5.0	6.5	5.0	4.0
	8:02½	Adrenalin m ii; true paradoxical phenomena in left eye	7.0	9.0	0.0	0.0
15th	11:20	Pseudo-paradoxical dilatation in left pupil	3.0	4.0	2.0	1.5
	11:23	Ether				
	11:38	Trace of pseudo-paradoxical dilatation in left pupil; wink reflex present	2.0	2.5	3.0	4.0
	11:38½	Adrenalin m ii; true paradoxical phenomena in left eye	8.0	10.0	0.0	0.0

than segment thoracic X, have preponderate functional relations with the dilator mechanism of the homolateral eye. This rule applies also to the thoracic and abdominal viscera in which certain embryological factors determine the source of the afferent nerve supply. In the primitive alimentary canal provision is made, through the relations of the simple tube to mesodermal substance, for a right- and left-sided afferent nerve supply. This primitive arrangement is the chief determinant of the preponderate afferent nerve supply (i.e., right- or left-sided as regards entry into the spinal cord) of the organs developed preponderately from one or other lateral aspect of the primitive gut. The afferents of the primitive gut do not stop at the median line but overlap on the ventral aspect so that each lateral aspect has a bilateral supply. As a consequence of this overlapping each lateral aspect of the primitive gut is served by afferent nerves that enter both sides of the spinal cord, the supply being derived preponderately from nerves that enter the cord on the homolateral side. This preponderance of afferent supply naturally extends to organs which develop preponderately from either lateral aspect of the primitive gut. In the light of these considerations the preponderance of the functional (afferent) relations of one lateral aspect of the gastro-intestinal tube, or of organs developed preponderately from one lateral aspect of the primitive gut, to the pupil dilator mechanism of one eye becomes intelligible. The crossed pupil dilator relationship of the skin and other organs receiving their afferent supply through the sacral, lumbar and lower thoracic nerves, depends perhaps mainly on the distance which the nociceptive pupil dilator paths entering the lower portion of the spinal cord have to travel before reaching the centers governing constrictor inhibition and sympathetic dilatation proper. The more caudalward the point of entry into the cord of an afferent nociceptive path the greater seems to be the possibility for it to finally become preponderately crossed before reaching the efferent dilator paths proper. As the liver and biliary system spring from a bud given off from the ventral aspect of the foregut it is not surprising to find that this system of organs has no fixed preponderance of functional relations with either eye. The pancreas has a twofold origin from the foregut, viz., *a*, a bud on the ventral aspect of the foregut, and *b*, one on the dorsal aspect. This organ also seems in the cat to have no fixed preponderance of functional relations with either eye. In the case of the spleen the evidence points to a fixed preponderance of left-sided afferent supply. The homolateral pupillary phenomena found after lesions of the renal cortex

indicate a preponderant homolateral afferent supply entering the cord for the most part cephalad of the 10th thoracic segment. On the other hand the contralateral pupillary phenomena found after lesions of the ureter and renal calyx point to a preponderant homolateral afferent supply that enters the cord for the most part caudad of the 10th thoracic segment. The key to the situation here is found in the embryological origin of these two sets of structures one of which, viz., the renal cortex, representing a secretory system, takes origin mainly from the cephalic half of the mesonephros which primarily (pronephros) extended from segment Cv to Liii and the other, viz., the renal calyx and ureter, representing the duct system of the mesonephros, from the caudal portion of the Wolffian duct near the point where this latter joins the cloaca. The origin of the renal duct system in a bud springing from the caudal portion of the Wolffian duct indicates that the afferent nerve supply of this system (ureter, calyx and collecting tubules) enters the spinal cord at a level preponderately caudad of the 10th thoracic segment as compared with the afferent nerve supply of the renal secretory system which enters the cord at a level preponderately cephalad of the 10th thoracic segment.

In its origin and development the genital duct system derived from the Müllerian ducts closely resembles the urinary duct system. It is not strange, therefore, to find a close similarity in the afferent nerve supply. In the genital duct system (uterine tubes, uterus, vagina, epididymis, etc.) the afferent supply is markedly caudad in origin as compared with that of the secreting system (ovary and testis). Hence the preponderant crossed pupillary relations of the duct systems as contrasted with the preponderant homolateral relations of the secretory systems. The sex glands are derived from the germinal epithelium of the genital ridge extending from the 6th thoracic to the 2nd sacral segment. It is stated, however, that the permanent sex glands in the human subject seem to be developed from the 4th lumbar to the 2nd sacral segments (4). At this point there is an apparent conflict between the physiological findings in the present paper and the embryological facts. More work, however, will undoubtedly bring complete harmony.

The bilateral afferent supply found in the alimentary system is even more emphatically evident in the circulatory system which primarily consists of paired bilateral systems of tubes. Even in its fully developed state the system remains tubular, the right and left heart, each with its own great artery (aorta and pulmonary artery), representing a

persistence of the primitive bilateral scheme. In view of these considerations, and of the cephalic position of the heart in relation to segment thoracic X, the preponderate homolateral relations of the cardiac and aortic afferent supply to the pupil dilator mechanism become intelligible.

The period of incubation (10 days) of the true paradoxical effects after right-sided thyroid gland injury shows that the pupillary effects were the result of lesion of afferent nerves of that organ. The contralateral pupillary effects found after unilateral lesions of the cecum and urinary bladder point to a preponderantly caudal afferent supply in all organs derived from the hind gut just as the homolateral pupillary effects found after unilateral thyroid, lung, heart and stomach lesions point to a preponderantly cephalic afferent supply in all organs derived from the foregut. Unilateral lesions confined to the parietal peritoneum do not seem to have any apparent influence in determining pseudo- or true paradoxical pupillary phenomena. The evidence here, so far as it goes, lends support to the contention of some of the older writers that the parietal peritoneum and the parietal pleura have no nociceptive nerve supply. Compare Head (5).

Paradoxical pupil dilator phenomena have an important clinical significance. This is especially true of pseudo-paradoxical dilatation (3). In the clinical recognition of this phenomenon it is necessary to bear in mind that one-sided somatic lesions below the level of the umbilicus (thoracic segment X) induce pseudo-paradoxical dilatation of the contralateral pupil whilst lesions above this level induce it in the homolateral pupil. It is also necessary to remember that an incubation or latency period of about 6 to 10 days is an essential characteristic of true paradoxical phenomena and that, after one-sided injury, pseudo-paradoxical dilatation occurs most commonly from the 1st to about the 6th day (pre-paradoxical stage) and from about the 25th day on (postparadoxical stage), depending on the location, nature and condition of the injury. During the true paradoxical stage, extending from about the 8th to the 25th day, pseudo-paradoxical dilatation may be absent although it is not unusual to find it present throughout the whole of the period of recovery. In visceral lesions, organs derived from the hind gut (colon, rectum, bladder, etc.) or preponderately from the caudal half of the Wolffian duct (ureteral system, epididymis, etc.) or of Müller's duct (uterine tubes, uterus, vagina, etc.) exhibit crossed pupillary relations, i.e., one-sided lesion of these organs induces pseudo-paradoxical dilatation in the contra-lateral pupil. Organs derived in part or in whole from the foregut or preponderately

from the cephalic half of the Wolffian body (renal secretory system) or of the germinal epithelium of the genital ridge (testis and ovary proper) exhibit uncrossed pupillary relations, i.e., lesions of one of these organs induces pseudo-paradoxical dilatation of the homolateral pupil. Taken in conjunction with the somatic regions of reflected pain and hyperalgesia which are more or less characteristic for each viscus, pseudo-paradoxical dilatation will prove itself to be a diagnostic sign of great corroborative value. The author has already found it most useful in the differential diagnosis of pathological conditions arising in the troublesome right upper and lower abdominal quadrants as well as in the diagnosis of calculus in the kidney or ureter, in gastric and duodenal ulcer, and in pulmonic, aortic and cardiac lesions. It should be remembered that in the presence of visceral or somatic lesions the related pupil may be smaller than its fellow when the patient is at rest and free from all pain. Anything, however, that evokes pain, e.g., active or passive movements, pressure, etc., will at once induce the characteristic pseudo-paradoxical dilatation. Attention to this point will convince the observer that the pupil inequality in any given case is not a mere accidental association. In paired organs such as the kidney owing to irregularities in development it is quite possible for the afferent nerve supply of one organ to be derived from nerves that preponderately enter the contralateral side of the spinal cord. In such conditions the pain associated with lesions of one kidney may be referred to the contralateral side. This is a familiar clinical phenomenon. So too in lesions confined to one lateral aspect of the appendix the typical associated pain and tenderness may be felt by the patient in the left instead of the right lower abdominal quadrant. The pain and tenderness associated with cardiac and aortic disease are preponderately left-sided for the embryological reasons stated above. In all these conditions, however, bilateral somatic tenderness, superficial and deep, can be readily demonstrated.

CONCLUSIONS

1. The skin over the hind quarter has preponderate functional relations with the pupil dilator mechanism of the contralateral eye whilst that covering the fore quarter, and either side of the head and neck, has preponderate functional relations with the pupil dilator mechanism of the homolateral eye.

2. The primitive cardio-vascular system and alimentary canal receive their afferent supply through nerves springing from either side of the spinal cord in such a way that the nerves from the right side of the

cord supply preponderately the right side of these structures whilst those from the left side of the cord supply preponderately the left side, a certain degree of overlap in the supply occurring in the ventral and ventro-lateral aspects of the structures in question.

3. Viscera developed from germ-buds springing preponderately from the right or left side of the primitive gut derive a preponderate afferent supply from nerves that enter the right or left side respectively of the spinal cord.

4. In viscera that remain tubular, e.g., the heart and great vessels (arch of aorta and pulmonary artery) and gastro-intestinal tract, the lateral aspect of each viscus receives a bilateral afferent supply although the preponderate supply is derived from nerves that enter homolateral aspect of the spinal cord.

5. One-sided lesion of organs receiving their afferent supply through nerves that preponderately enter the spinal cord above segment thoracic X, e.g., lungs, heart, stomach, etc., is attended by pseudo- and true paradoxical pupil dilatation in the homolateral eye, whereas one-sided lesion of organs receiving their afferent supply through nerves that enter the cord preponderately below segment thoracic X, e.g., the colon, uterus, bladder, ureter, epididymis, etc., is attended by pseudo- and true paradoxical pupil dilatation in the contralateral eye.

6. Pseudo-paradoxical dilatation especially when taken in conjunction with somatic pain and tenderness (reflected pain and hyperalgesia) furnishes a useful corroborative sign in the diagnosis of visceral lesions.

7. Owing to the bilateral afferent nerve supply of the viscera lesion of any viscus is usually attended by bilateral somatic tenderness (reflected) although the typical pain is invariably one-sided and usually but not universally homolateral.

8. The homolateral pupillary phenomena associated with lesions of the secretory portions of the genital and urinary systems stand in sharp contrast to the contralateral pupillary phenomena associated with lesions of the conducting channels of these systems.

9. The evidence adduced in these studies gives no indication that the parietal peritoneum has a nociceptive nerve supply.

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BIOLOGICAL FOOD TESTS

III. CHANGES IN VITAMINS A AND B OF THE GLOBE ARTICHOKE DUE TO VARIOUS CANNING AND DRYING PROCESSES

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Received for publication May 16, 1923

The flowering head of the globe or French artichoke, *Cyanara scolymus*, has of late years begun to be used fairly commonly as a salad vegetable throughout the United States, and is particularly freely used in California. Very little study of its composition or nutritive properties has been carried out, only two articles being reported from American laboratories. The first of these by Wiley (1) dealt with the question of the use of adulterants in canned artichoke hearts, and the second, by Okey and Williams (2), with the presence of inulin among the carbohydrates of the whole head.

Composition of the globe artichoke. The latter paper contained also a proximate analysis of the artichoke head which is of interest in connection with the present report. The figures given by these authors for the raw whole flowering head of artichoke are as follows:

	per cent
Moisture.....	85.5
Ash.....	1.1
Crude fiber.....	2.4
Protein.....	2.8
Ether extract.....	1.4
Total carbohydrate.....	6.8

This analysis does not represent the content of those parts of the artichoke which are actually eaten, since a large part of the fibrous tissue of the petals must be discarded. It seemed of interest therefore to the writers to determine more clearly the composition of the edible portion of the artichoke. This was accomplished by boiling the whole heads in water for one to one and one-half hours as is the usual custom, draining, and scraping off the soft part of the leaves and the heart. This edible pulp was then dried at 70°C., ground and then subjected to

the usual proximate analysis (3). The average results obtained from several analyses were as shown in table 1.

When these results are compared with those quoted above for the raw whole artichokes after allowance has been made for the difference in water content, it will be found, as might be expected, that the dried edible portion contains a considerably higher proportion of carbohydrate and a lower amount of protein and ether extract than the whole flowering head.

Since the actual quantity of solid nutrients obtained from the artichoke as usually eaten is necessarily so small, and since even of these a considerable proportion as shown by Okey and Williams (1) consists of inulin, approximately 60 per cent of available carbohydrate, which is probably not digested in the animal body, it seemed worth while to attempt to discover the vitamin content of this vegetable.

TABLE 1

Composition of dried globe artichoke, E.P.

Moisture.....	6.0
Ash.....	7.3
Protein.....	12.3
Ether extract.....	2.6
Total carbohydrate.....	71.8

Effect of canning and drying upon vitamin content. Another problem, which it was thought could be attacked at the same time, was that of the comparative effect upon vitamins A and B of two types of canning process much in use at present, as well as of the usual low temperature drying of such samples.

The stability of vitamin B to heat and oxidation. A considerable volume of research has been carried out on the question of the stability of the various vitamins to heat and oxidation. Some of these studies upon vitamin B were made with the technique of the yeast growth method proposed by Williams (4), and since the accuracy of this method has been seriously questioned, are now of doubtful value. The work of Miller (5) on carrots and beans, of Whipple (6) on cabbage and onions, and of Eddy and Stevenson (7) on a variety of fruits and vegetables, falls in this class. In all these investigations ordinary boiling for from 30 to 60 minutes was found to have practically no effect upon the vitamin B potency of the foods used. Osborne and Mendel (8) using the rat feeding method with pasteurized milk concluded that vitamin B "is stable

at temperatures below 100 degrees, and perhaps even at considerably higher temperatures." Emmett and Luros (9) using pigeons showed that the antineuritic vitamin in unmilled rice was totally destroyed by heating for 2 and 3 hours in the autoclave at 120 degrees and 15 pounds pressure, but that the rat growth-promoting vitamin B on the other hand was little affected under these conditions. This difference they interpreted to indicate separate identities of the two water-soluble vitamins. In all the studies reviewed above moist heat was used.

The effect of dry heat or desiccation on vitamin B was investigated by Osborne and Mendel (10), who concluded that the efficiency of fruit juices in this respect was not lost by "suitable modes of desiccation." Karr (11) reported work with yeast using dogs as the experimental animals. He found that redrying moistened brewery yeast at 108 degrees for 72 hours had little effect upon its vitamin B content, while the same material when first autoclaved from 3 to 4 hours at 120 degrees and 15 pounds pressure was found to have lost a considerable part of its potency.

There would appear therefore to be little unanimity upon the question of the relative destruction of vitamin B in foods by moist heat under ordinary pressure or in the autoclave, and by the heightened temperatures used for desiccation. The importance of an adequate answer to this question can hardly be over-estimated, however, in view of the increasing use of canned and dehydrated foods in all parts of the world.

The stability of vitamin A to heat and oxidation. Although the stability of vitamin A to heat and oxidation has been the subject of a number of investigations, considerable uncertainty still exists as to quantitative reduction by these conditions of this substance or property, particularly in vegetable sources of the vitamin. The early work of Osborne and Mendel (12) and of McCollum and Davis (13) chiefly with steam and water treatment of butter, seemed to show vitamin A relatively thermostable. Steenbock, Boutwell and Kent (14) were the first to show that partial destruction occurred in butter upon treatment with steam, carbonated water or air, and soon afterward the work of Drummond and Coward (15), Hopkins (16) and Zilva (17) seemed to prove conclusively that destruction of vitamin A occurs rapidly at high temperatures only when oxidation accompanies the treatment. Hopkins used autoclaved and aerated butter, Drummond and Coward minimal doses of aerated and unaerated butter, and Zilva butterfat and codliver oil affected by the ozone treatment incident to exposure to ultraviolet rays.

Only a few studies have been made upon the effect of oxidation and heat on the vitamin A in plant materials. Steenbock and Boutwell (18) found no measurable effect upon vitamin A as a result of autoclaving for 3 hours at 15 pounds pressure and then air-drying a variety of roots, tubers, grains and leaves. Osborne and Mendel (19) report high efficiency in alfalfa, clover, tomatoes and spinach following air-drying at 60 degrees, but without offering corresponding figures for the fresh untreated vegetables. Indeed, so far as is known to the writer, no conclusive evidence whatever has been offered by this comparative method as to the stability of vitamin A in plant materials toward either heat or oxidation.

The plan of the present investigation was to compare the potency with respect to both vitamins A and B of freshly cooked artichoke with that of samples of the same vegetable after it had been canned by two different processes, and desiccated by the usual low temperature air-drying method. No attempt was made to eliminate all air from the jars used in the canning experiments, but in all cases the water extract produced during the cooking and canning was retained and fed along with the solid parts of the vegetable. In this way the normally combined effects of heating and oxidation were measured together, while mechanical loss of vitamins by water extraction was avoided.

The types of canning processes chosen for comparison were *a*, the usual fractional sterilization method advocated for home canning of vegetables; and *b*, the autoclave or pressure canning process used in commercial canneries.

In the first method the vegetables are heated in a boiling water bath for one hour on three consecutive days on the theory that successive spore crops thus allowed to develop into less resistant vegetative forms may be completely destroyed. The total exposure of the vegetables to heightened temperatures is somewhat variable under these conditions, since the water in the bath may be raised to the boiling point at varying rates, and since the maximum temperature attained by the vegetables depends upon solidity of pack, amount of water, size of jar, etc. Some of these variables have been studied by Castle (20), Denton (21) and others.

Methods of canning used. A uniform procedure was adopted for all of the canning carried out in this study, and the total quantity of artichokes needed for the feeding experiments canned at one time. The methods adopted to represent the two processes were as follows:

a. Cold-pack fractional sterilization. The artichokes were cut into quarters, packed uniformly in pint size glass jars, with loosely adjusted covers, with no added water, completely submerged in a water bath which was brought to the boiling point at a constant rate, and the boiling continued one hour. The jars were then immediately removed from the bath, covers tightened, and jars allowed to cool at room temperature. On the two succeeding days the process was repeated, except that the covers were not again loosened.

b. Steam pressure sterilization. The artichokes were packed in the same fashion as for the fractional sterilization, and were processed for one hour at 15 pounds pressure, in a large steam autoclave.

It will be noted that in the first process the artichokes were submitted to a possible maximum temperature of 100° for a total of 180 minutes, and to varying temperatures between room temperature and 100° for some 6 hours, the total period of heating of the water bath to boiling, and of cooling of the jars after each partial sterilization. In the second process the vegetables were submitted to a possible maximum temperature of 121° for 60 minutes, and to varying temperatures between this maximum and room temperature for approximately 2 hours during the preliminary heating and subsequent cooling. It was thought that the question of the relative importance of time of heating as compared with temperature reached in diminishing the vitamin content of vegetables might be approached through feeding experiments using the artichokes canned in these two ways.

Studies were made of the vitamin B and vitamin A values of both types of canned artichoke, and these results compared with those obtained by the use of dried artichoke and freshly boiled artichoke.

Methods of testing for vitamins A and B. The methods used in testing the vitamin A and vitamin B content of all the artichoke preparations were the same as those previously described (22). In all cases only the edible portion of the artichoke was fed together with any liquid found in the jars. The portions of canned vegetable fed were weighed wet. The water content was determined in several samples of freshly boiled, pressure canned, and fractionally canned artichoke, and found to vary within the same limits in all these samples, 82 to 85 per cent.

Vitamin A in pressure canned artichoke. Four young rats were fed from time of weaning on the standard vitamin A-free basal diet plus 0.3 gram of dried brewery yeast as a source of vitamin B, and 4 grams of wet edible portion of pressure canned artichoke as source of vitamin A. This amount of wet vegetable contained 0.6 gram of dry

material. All of these animals grew satisfactorily, their growth curves in all cases being, in fact, steeper than those shown by litter mate controls fed a similar diet but with 10 per cent butterfat instead of artichoke.

Vitamin A in fractionally canned artichoke. A similar group of four rats was treated in precisely the same manner, except that 4 grams of fractionally canned artichoke were fed as sole source of vitamin A, and with nearly the same results, as may be seen in chart 1. Growth was excellent in all cases, and it would be difficult indeed to adjudge any difference between the two types of canned vegetable. A considerably lower level of dosage must be employed before any slight differences can be detected.

Vitamin A in freshly cooked artichoke. Four animals were used in a similar test of the vitamin A content of freshly boiled artichoke. The vegetables were cooked without added water in glass jars in a boiling water bath for 90 minutes. This is the usual length of time of boiling employed in the preparation of artichokes for the table. At first 4 gram moist edible portions were fed as source of vitamin A, and this dose gradually decreased to 2 grams, or 0.3 gram dry material. When the dose fell below 2 grams the body weight increases of the animals fell off perceptibly. This quantity then was adopted as representing the probable minimum upon which normal growth could be secured. The maximum destruction of vitamin A by either process of canning as compared with a single boiling would appear, therefore, to be near 50 per cent.

Vitamin A in dried artichoke. Fresh artichokes boiled 90 minutes were scraped to obtain the edible portion, and this material dried rapidly at 70°. The resulting mass, the composition of which is given in table 1, was finely ground and used as source of vitamin A in a curative test upon four young rats. These animals upon vitamin A-free diet had developed extreme cases of the eye disease characteristic of vitamin A deficiency, rapidly falling weight, and in at least two cases, rats 105 and 118, were obviously moribund. One gram of the dried artichoke was administered daily, and within ten days all the rats showed normal eyes, excellent appearance of nutrition, and rapid increase in body weight. The dosage of dried artichoke was decreased to 0.5 gram in two cases, but growth continued uninterruptedly at a normal rate.

A comparison of results obtained with these four artichoke preparations as sources of vitamin A is shown in table 2, and typical growth curves for the animals used are shown in chart 1.

Vitamin B in pressure canned and fractionally canned artichoke. Similar groups of four animals each were used in testing for vitamin B in the two types of canned artichoke by the protective method, using a dose of 4 grams of edible portion of the vegetable in each case. The usual basal diet containing 10 per cent of butterfat was given, but no additions of dried yeast. Growth was obtained in all cases, but somewhat below the normal rate. The animals could not be induced to eat appreciably more than the 4-gram amount of artichoke, so that it was impossible to determine the optimum level of this food for vitamin B.

Upon careful superposition and comparison of the growth curves obtained with one another, and with optimum control curves, there may be discerned a definite advantage of the group fed the pressure canned

TABLE 2
Vitamin A content of the globe artichoke

PREPARATION TESTED	MAXIMUM DOSE TESTED		METHOD USED	NUMBER OF RATS	LENGTH OF FEEDING PERIOD	RESULT
	Moist	Dry				
	grams	grams				
Pressure canned artichoke, E. P.	4	0.6	Protective	4	20	Normal growth
Fractionally canned artichoke, E. P.	4	0.68	Protective	4	20	Normal growth
Freshly cooked artichoke, E. P.	2	0.36	Protective	4	20	Normal growth
Dried artichoke	0.5	0.47	Curative	3	20	Normal growth

vegetable over those fed the fractionally canned. The growth of the former group took place practically at the same rate as that of the group receiving similar amounts of freshly cooked artichoke, but those receiving the fractionally canned food exhibited a more retarded rate. Typical growth curves illustrating these points are shown in chart 2.

As indicated, therefore, in table 3, there would appear to be a definite though slight advantage in the use of steam pressure canning above the longer fractional process in the preservation of the vitamin B content of globe artichokes. Whether this advantage is present under the same conditions in the case of other green vegetables, is naturally a question impossible to answer without further experiment.

Vitamin B in freshly cooked artichoke. By means of the same technique it was ascertained that nearly normal growth could be obtained

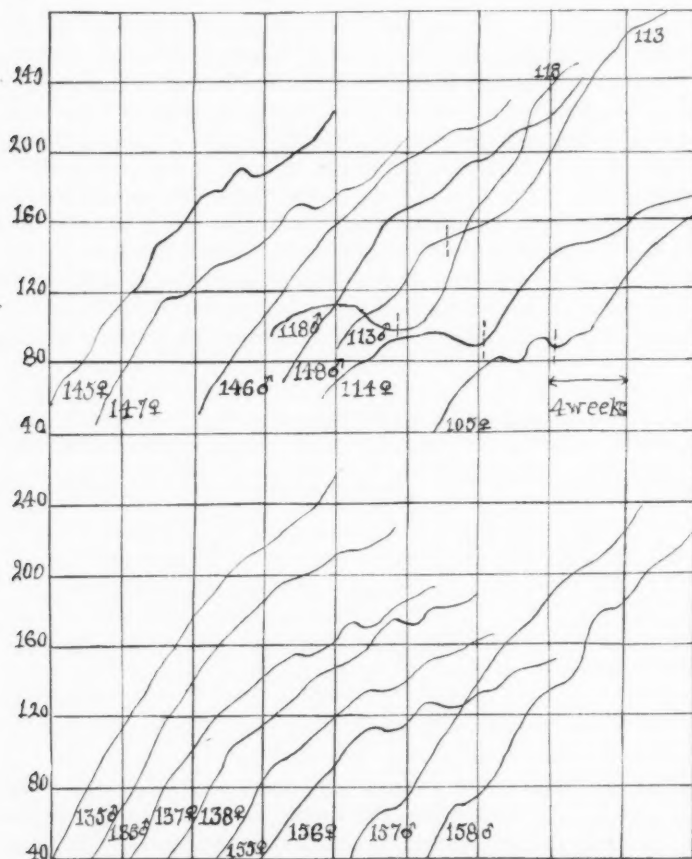


Chart 1. Illustrative growth curves of rats fed artichoke as source of vitamin A.

Basal diet—casein, 18; agar, 2; salts, 4; dextrin, 56; crisco, 20—and 0.3 gram dried brewery yeast daily given in each case in addition to source of vitamin A, administration of which was begun at dotted line in curative experiments.

Rats 135, 136, 137, 138 were fed 4 grams pressure canned artichoke, weighed moist.

Rats 155, 156, 157, 158 were fed 4 grams fractionally sterilized artichoke, weighed moist.

Rats 145, 146, 147, 148 were fed 2 grams freshly cooked artichoke, weighed moist.

Rats 105 and 113 were fed 0.5 gram dried artichoke from dotted line as a curative measure, and rats 114 and 118, 1 gram dried artichoke from dotted line.

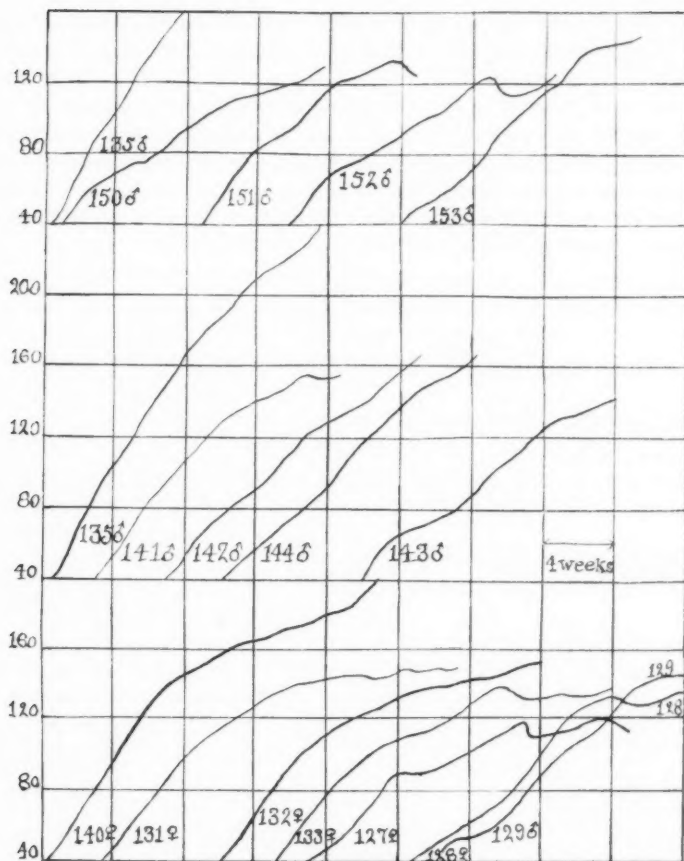


Chart 2. Illustrative growth curves of rats fed artichoke as source of vitamin B. Basal diet containing 10 per cent of butterfat, instead of a corresponding amount of crisco, was given in addition to the source of vitamin B.

The curves of rats 140 and 135 represent normal growth curves for female and male rats respectively.

Rats 150, 151, 152, 153 were fed 4 grams fractionally sterilized artichoke, weighed moist.

Rats 141, 142, 143, 144 were fed 4 grams freshly cooked artichoke, weighed moist.

Rats 131, 132, 133 were fed 4 grams pressure canned artichoke, weighed moist.

Rats 127, 128, 129 were fed 2 grams dried artichoke.

by the use of 4 grams of freshly cooked artichoke as source of vitamin B. When the dosage was lowered to 3 or 2 grams, however, the weight increases fell off perceptibly.

Vitamin B in dried artichoke. Dried artichoke administered in the same manner in doses as large as 2 grams did not provide sufficient vitamin B for normal growth of young rats. No definite symptoms of vitamin B deficiency were observed, but over a period of 5 months the growth curves exhibited by all the animals fed were some 30 per cent flattened below normal.

TABLE 3
Vitamin B content of the globe artichoke

PREPARATION TESTED	MAXIMUM DOSE NEEDED		METHOD USED	NUMBER OF RATS	LENGTH OF FEEDING PERIOD	RESULT
	Moist	Dry				
	grams	grams			weeks	
Pressure canned artichoke, E.P.	4	0.6	Protective	4	20	Growth about 10 per cent below normal rate
Fractionally canned artichoke, E. P.	4	0.68	Protective	4	20	Growth about 30 per cent below normal rate
Freshly cooked artichoke, E.P.	4	0.72	Protective	4	20	Growth about 10 per cent below normal rate
Dried artichoke	2	1.98	Protective	3	20	Growth about 30 per cent below normal rate

SUMMARY

1. It has been shown that the edible portion of the globe or French artichoke, when freshly boiled, is a comparatively rich source of vitamin A, 0.36 gram of dry material daily providing for normal growth in rats.

2. The same preparation of artichoke is a considerably less valuable source of vitamin B, 0.72 gram of dry material providing for growth at less than normal rate.

3. Processing the artichoke under 15 pounds pressure for 60 minutes decreases the vitamin A value approximately 50 per cent, 4 grams moist or 0.6 gram dry material being required for normal growth. Processing

under atmospheric pressure in a boiling water bath on 3 successive days for 60 minutes each time, had apparently the same effect upon the vitamin A of the artichokes, since approximately the same amounts were needed to produce normal growth as were used in the case of the pressure canned samples.

4. The effect of the two types of canning upon the vitamin B of the artichoke could not be determined quantitatively, since the animals refused to eat larger doses than the 4 gram moist portions. It was plain, however, that the longer fractional sterilization produced a somewhat greater destruction of this vitamin than did the pressure processing, since upon similar doses the rats receiving the pressure canned artichoke made better growth than those upon the fractionally canned, and indeed approximated closely that shown by the group fed the freshly cooked vegetable.

5. The edible portion of the artichoke, dried at 70°C., produced excellent growth and removal of vitamin A deficiency symptoms when fed in doses of 0.47 gram as sole source of vitamin A. When fed as sole source of vitamin B doses as high as 2 grams daily failed to promote growth at the normal rate.

6. The destruction by canning and drying of vitamin A in this green vegetable appears to be relatively small, and to be effected somewhat indifferently by varying temperatures.

7. The destruction of vitamin B by canning and drying appears to be relatively rapid, and to be more completely effected by long exposure to relatively low temperatures as in the drying and fractional canning processes described, than by shorter exposure to higher temperatures as in the pressure process used.

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STUDIES OF THE PATHOGENESIS OF TETANY

III. EXCITING FACTORS IN EXPERIMENTAL TETANY IN DOGS

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Received for publication May 17, 1923

In the previous reports (1) of this study on the pathogenesis of tetany, a description has been given of a method which will prevent the onset of tetany or depression in dogs following the complete removal of the parathyroid glands and will permit these animals to survive indefinitely and in good condition. The principle of the method involves the prevention of bacterial proteolysis in the intestines by diet. When this is done the parathyroid glands may be removed without the appearance of the usual post-operative tetany or depression. On the basis of these experiments the conclusion has been suggested that the function of the parathyroid glands is in part to protect the body from intoxication of intestinal origin. The tetany or depression which occurs when the glands are removed is due to the accumulation of various toxic substances in the blood, most of which were produced in the intestinal tract by the proteolytic action of the intestinal bacteria. When this source of toxemia is checked by dietary treatment, there is not sufficient toxemia to cause untoward symptoms, except under the special conditions detailed later in this report. When the parathyroidectomized animals have been kept free from tetany for periods of three to six weeks, by dietary management, some readjustment occurs in the body so that these animals can take ordinary mixed diets without developing tetany or depression. There is a gradual increasing resistance of the body to the tetany toxins, which seems to involve a vicarious detoxicating activity on the part of the liver. The readjustment is gradual, however, and is perhaps never complete. The animals remain in a state which strikingly resembles latent tetany and certain other spasmodic affections in man. An opportunity has thus been afforded to note many diverse exciting factors of tetany in these animals.

Tetany produced by feeding meat. As a routine procedure dogs have been placed on a diet of bread, milk and lactose until the feces gave evidence that bacterial putrefaction in the intestines had been checked. The thyroid and parathyroid glands were then removed. Following the operation the milk and lactose were given by the stomach tube in cases where the food was refused. Under such treatment the dogs remained in good condition and it became a question if these were not cases where accessory parathyroid tissue was present. To test this possibility the animals were offered meat, usually in the third week after the operation. The meat was readily taken and in every case on the following day toxic symptoms appeared ranging all the way from tremors and spasticity to the most violent convulsions seen in recently operated animals. The following is a typical experiment:

Dog 30. Large female dog; weight 18 kgm.

May 13, 1922. Diet, white bread and milk ad lib. and lactose, 60 grams per day. May 19th. Feces liquid, odorless, acid to litmus, fecal bacteria predominantly aciduric. Complete thyro-parathyroidectomy.

May 20th-29th. Condition good. No evidence of tetany or depression. Three hundred cubic centimeters of milk and 300 cc. of lactose solution (20 per cent) given daily by stomach tube.

May 30th-June 6th. Diet, bread, milk and lactose (60 grams). The dog has developed a marked enophthalmos, blepharospasm, and the nictitating membrane is drawn over the eye. There are occasional fine tremors in the neck muscles.

June 7th-15th. Diet, white bread, milk and lactose. Condition improved. No tetany. Weight 16 kgm.

June 16th. Condition good. No evidence of tetany. Offered two pounds of meat which was eaten voraciously.

June 17th. The animal developed a violent attack of typical parathyroid tetany followed by extreme depression. Death occurred in a second attack of tetany four hours later. Autopsy failed to reveal any accessory parathyroid tissue.

In other experiments the meat was given at longer intervals from the operation, and in many cases after the animals had been able to take a mixed diet without developing toxic symptoms. In these animals it was noticed that *putrid meat* was more likely to occasion toxic symptoms than an equal quantity of fresh meat. Where an exclusive meat diet was given to dogs that had so far recovered from the operation that they could take the stock diet without tetany, the symptoms that occurred were relatively mild and not always characteristic of acute tetany. The enophthalmos and blepharospasm described by Luckhardt (2) and Paton (3), often inducing a severe conjunctivitis,

were commonly seen. One animal developed coarse jerking movements of the head, which strikingly resembled those seen in paralysis agitans in man. The movements were distinctly worse when the animal was resting and usually disappeared when moving about or eating.

Tetany produced by constipation. Constipation or stasis is definitely a factor in inducing tetany in parathyroidectomized dogs. The prevention of post-operative tetany or depression for the first four weeks by the diet of bread, milk and lactose is entirely dependent upon securing free and liquid or semi-solid bowel movements. The diarrhea is doubtless the result of the intestinal irritation by the acid products resulting from bacterial action on the lactose. Whenever, as a result of persistent vomiting, it is impossible to keep the milk and lactose solution in the stomach and the fluid stools cease, symptoms of tetany usually appear. These can be controlled by the use of enemas. If the tetany intoxication has become marked there is an almost complete paralysis of gastro-intestinal movements, which along with the anorexia and vomiting forms a vicious circle which is very difficult to control. The intestinal stasis favors the production and absorption of intestinal poisons and these in turn increase the paralytic ileus. Dragstedt and Cannon (4) found that even when animals were kept on an aciduric diet, the production of a stasis or a complete obstruction invariably led to a predominance of proteolytic intestinal bacteria.

Tetany produced by high temperatures. The following experiment indicates that fatal tetany may be induced in a dog, that has recovered from complete parathyroidectomy, by exposure to high temperatures.

Dog 27. Large male dog; weight 19 kgm.

March 1, 1922. Diet, white bread and milk ad lib. and lactose, 80 grams per day.

March 10th. Feces liquid, odorless, acid to litmus, fecal bacteria aciduric in type. Complete thyro-parathyroidectomy.

March 11th-13th. Condition good. No tetany.

March 14th. Tremors in temporal muscles, marked hyperpnea, slight clonic convulsions: 500 cc. of milk and 400 cc. lactose solution (22 per cent) given by stomach tube.

March 15th-April 1st. Condition good. No tetany.

April 2nd. Animal given stock diet of meat, bread and vegetables.

April 3-10th. Condition good. No tetany.

May 11th. There was noted a profuse salivation, hyperpnea, spasticity of leg muscles, and tremors in temporal muscles. The animal was constipated and groaned on attempting bowel movements. Condition relieved by enemata.

May 12th. Recovery complete. No tetany.

July 1st. Transferred to attic as cured.

- July 10th. Animal found in violent tetany. The room was very warm (95°F.), humid and poorly ventilated. Nothing unusual had been done and the food was the same as on previous days. Seven hundred cubic centimeters Ringer's solution were given intravenously.
- July 11th. Recovery. No tetany but dog refused food.
- July 12th. Animal depressed. Refused food. No tetany. Seven hundred cubic centimeters Ringer's solution intravenously.
- July 13th. Dog found dead in cage. Autopsy failed to disclose any accessory parathyroid tissue.

Since this experiment it has been observed on four different occasions that an exceedingly hot day or, through faulty regulation a high temperature in the room would excite tetany in recovered parathyroidectomized dogs, and this, in our opinion, independent of other possible factors.

Tetany excited by muscular exercise. It has been repeatedly observed in several different animals, at considerable periods after removal of the parathyroids, that muscular exercise would bring on transient symptoms of tetany in dogs otherwise free, and would invariably augment the symptoms when these were present in mild form. In the following experiment severe convulsions were induced by muscular exercise in a dog that had been free from tetany for a long period. The experiment is also interesting because the dog was pregnant when operated. This factor will be referred to later.

Dog 36. Small female dog; weight 9 kgm.

- July 15, 1922. Animal is about six weeks pregnant. Diet, white bread and milk ad lib. and lactose 60 grams per day.
- July 21st. Feces liquid, odorless, acid to litmus; fecal bacteria predominantly aciduric. Complete thyro-parathyroidectomy.
- July 22nd. Animal was in tetany (salivation, hyperpnea, clonic convulsions). Seven hundred cubic centimeters of lactose solution (20 per cent) given by stomach tube in morning. Six hundred cubic centimeters of Ringer's solution given intravenously in afternoon. Recovery.
- July 23rd. No tetany. Condition fairly good. Four hundred cubic centimeters lactose solution (20 per cent) given by stomach tube.
- July 24th. Slight fibrillary tremors in temporal muscles. No convulsions. Voluntarily drank some milk and lactose solution.
- July 25th. Slight depression. Few transient tremors. Four hundred cubic centimeters of lactose solution (20 per cent) given by stomach tube.
- July 26th. 8:00 a.m. Slight fibrillary tremors in temporal muscles.
9:00 a.m. Marked hyperpnea, spasticity, tonic and clonic convulsion. Five hundred cubic centimeters of Ringer's solution plus 1 gram of calcium lactate given intravenously.
10:00 a.m. Convulsions subsided. Four dead pups were born. No tetany.
- July 27th and 28th. No tetany. Slight depression. Refused food.

- July 29th. Violent tetany (tonic and clonic convulsions). Five hundred cubic centimeters Ringer's solution given intravenously; 500 cc. lactose solution (20 per cent) given by stomach tube. Tetany relieved.
- July 30th-Sept. 1st. Condition good. No evidence of tetany.
- Sept. 2nd. Put on stock diet of bread, meat and vegetables.
- Sept. 3rd.-Nov. 3rd. Condition good. No evidence of tetany.
- Nov. 4th. Slight spasticity. Put on diet of bread, milk and lactose.
- Nov. 5th. Condition good. No tetany.
- Nov. 6th. Animal seemed normal and very lively. It jumped from the cage, ran excitedly about the room for two or three minutes, and then suddenly fell on its side in violent convulsions. These were first tonic and accompanied by marked hyperpnea and profuse salivation. The tonic contractions were gradually succeeded by clonic contractions, and these became gradually slower and weaker, finally stopping altogether. The animal immediately regained her feet and continued running about the room showing little or no evidence of depression.
- Nov. 7th-29th. Condition good. No evidence of tetany.
- Nov. 30th. Returned to stock diet.
- Dec. 1 to Jan 1, 1923. Condition excellent. Weight 10 kgm. During this time the animal was very lively and excitable. Faint tremors appeared in the temporal and leg muscles following exertion.
- Jan. 2nd. Found dead in cage, apparently dying in severe tetany. The tongue was bitten between the locked teeth. The room was very warm, due to faulty steam regulation and it is probable that the heat was a factor in inducing the fatal tetany.

The striking similarity of the attack occurring on November 6th to the Grand Mal attacks seen in human idiopathic epilepsy is very suggestive. Several of the recovered animals have developed similar seizures, which in the rapidity of onset and the comparative freedom from symptoms following the attack more nearly resemble human epilepsy than any other condition.

Tetany induced by oestrus and sexual excitement. Confirming the observations of Luckhardt (5), it has been repeatedly observed that completely parathyroiectomized dogs, who have been kept free from tetany for long periods by dietary treatment, may develop all symptoms of tetany during the period of heat or oestrus. The onset of the tetany is very gradual. At first there is often a slight enophthalmos and blepharospasm. Other animals develop spasms of the mouth muscles, brought on particularly by some slight excitement. On approaching the cage the animal manifests all the usual signs of welcome (wags tail, wriggles, lies on back) except that the lips are drawn from the teeth in a snarl. In a day or two the condition gets worse and if the animal is on a fairly high meat diet, fatal tetany may ensue. The following experiment illustrates the recurrence of tetany during the period of heat and the production of severe tetany by the act of copulation.

Dog 38. Adult, female dog. Weight 10 kgm.

- July 20, 1922. Diet, white bread and milk ad libitum, lactose 60 grams per day.
Aug. 2nd. Feces liquid, odorless, acid to litmus. Complete thyro-parathyroidectomy.
Aug. 3-4th. Condition good. No evidence of tetany.
Aug. 5th. Severe tetany (tonic and clonic convulsions). Five hundred cubic centimeters of 20 per cent lactose solution given by stomach tube. In a short time there was a profuse watery bowel movement. Tetany subsided.
Aug. 6th-7th. Condition good. No tetany.
Aug. 8th. Occasional jerking movements of forelegs.
Aug. 17th. Slight tetany (hyperpnea, salivation, spasticity, tremors in temporal and neck muscles). Very warm day. Five hundred cubic centimeters Ringer's solution given intravenously. Recovery.
Aug. 18th-Oct. 30th. Condition good. No evidence of tetany.
Oct. 31st-Nov. 9th. Dog is in heat. Has been observed in copulation. During this period there has developed a marked enophthalmos and blepharospasm but no convulsions.
Nov. 10th. During copulation with male there was marked excitement and spasticity but no convulsions. Immediately after copulation a second male was brought in. During copulation with the second male a violent convulsion occurred (tonic and clonic generalized convulsions, salivation and hyperpnea). Five hundred cubic centimeters Ringer's solution given intravenously; 500 cc. of lactose solution (20 per cent) given by stomach tube.
Nov. 11th-13th. Condition good. No tetany. No longer in heat.
Nov. 14th. Slight fibrillary tremors in jaw muscles. Can be thrown into violent tetany by excitement or muscular exercise.
Nov. 15th-Dec. 22nd. Condition good. No evidence of tetany.

Tetany induced by infection. It was early noted that if no infection occurred at the site of operation it was much easier to keep the animals alive and free from tetany after parathyroidectomy than if the wound became suppurative. In the following experiments fatal tetany was produced long after the animals had recovered from the operation, in the one case by an extensive skin infection (mange) and in the other by a suppurative cellulitis.

Dog 22. Large, adult, male. Weight 16 kgm.

- Jan. 13, 1922. Diet white bread and milk ad libitum, and lactose 50 grams per day.
Jan. 24th. Feces liquid, odorless, acid to litmus. Complete thyro-parathyroidectomy.
Jan. 25th-26th. Condition good. No tetany.
Jan. 27th-31st. Daily tremors and spasticity. No convulsions. Five hundred cubic centimeters milk and 400 cc. of lactose solution (20 per cent) given daily by stomach tube. This increased the diarrhea and relieved the tetany symptoms.

- Feb. 11th. Condition good. No tetany.
- Feb. 12th. Put on stock diet of bread, meat and vegetables.
- Feb. 13th.-March 14th. Condition good. No tetany.
- March 15th-Oct. 1st. Condition good. Gaining in weight. Has been repeatedly observed in copulation. It has been noted that at times, particularly after meals rich in meat, there appear jerking movements, twitchings, and even slight tetanic convulsions of the muscles of the jaws, mouth and neck. These at times bear a striking resemblance to the movements seen in paralysis agitans.
- Oct. 2nd. Badly infected with mange.
- Oct. 3-15th. Progressive loss of weight and strength. At times the animal staggers about, then falls to the ground, becoming very rigid, then relaxes and appears moribund. Recovery from these attacks is gradual. In the interval the animal appears in fairly normal condition. When at rest the coarse jerking movements of the head and shoulders, previously noted, are very prominent.
- Oct. 16th. Depression, weakness and convulsive attacks more frequent and severe.
- Oct. 18th. Found dead in cage. From position of body apparently did not die in tetany. Aside from the extensive mange, which covered almost the entire body, no abnormalities were found at autopsy. There was no trace of thyroid or parathyroid tissue.

Dog 31. Adult, female. Weight 22 kgm.

- May 6 1922. Diet white bread and milk ad libitum, and lactose 80 grams per day.
- June 6th. Feces liquid, odorless, acid to litmus, fecal bacteria predominantly aciduric. Complete thyro-parathyroidectomy.
- June 7th-12th. Condition good. Appetite good. No evidence of tetany.
- June 13th. Condition good. No tetany. Fed about 1½ lb. lean meat.
- June 14th. Slight muscular tremors. Five hundred cubic centimeters of 20 per cent lactose solution given by stomach tube.
- June 15th-18th. Recovery. No tetany but animal seems somewhat depressed. Appetite good.
- June 19th. Was given two pounds of cooked meat which was devoured eagerly.
- June 20th. Violent tetany (hyperpnea, salivation, tonic and clonic generalized convulsions). Seven hundred cubic centimeters of Ringer's solution were given intravenously. Tetany subsided.
- June 21st. Depression. No tetany. Nine hundred cubic centimeters Ringer's solution given intravenously.
- June 22nd-July 2nd. Condition precarious throughout. There have been many violent attacks of tetany followed by severe depression. Twice the respiration stopped due to spasms of the respiratory muscles and glottis, requiring artificial respiration. Nine hundred cubic centimeters of Ringer's solution given intravenously each day, daily enemas, and 400 cc. lactose solution (20 per cent) given by stomach tube.
- July 3rd. Dog now free from tetany. Feces liquid and odorless. Appetite good. Weight 18 kgm.

- July 4th-Sept. 1st. Condition good. No tetany. Gaining in weight. Diet white bread, potatoes, milk and lactose.
- Sept. 2nd. Severe tetany. Exciting factor unknown. Nine hundred cubic centimeters Ringer's solution given intravenously.
- Sept. 3rd-Nov. 22nd. Condition good. No tetany.
- Nov. 23rd. Condition good. Weight 24 kgm. Given stock diet of bread, meat and vegetables.
- Nov. 24th-Dec. 10th. Condition good. No tetany.
- Dec. 10th-16th. The joints in the forelegs have become swollen and painful and numerous small communicating subcutaneous abscesses have developed in the legs and shoulders. Coincident with the development of the infection, symptoms of tetany such as enophthalmos, blepharospasm and spasticity have appeared.
- Dec. 17th. Severe tetany. Eight hundred cubic centimeters Ringer's solution given intravenously. Recovery.
- Dec. 18th. Spasticity, tremors, but no convulsions. Large suppurating sinuses have developed in both shoulders. Eight hundred cubic centimeters of 20 per cent lactose solution given by stomach tube.
- Dec. 20th-28th. Alternate severe tetany and depression. Treatment with lactose and Ringer's solution daily. Infection has become deeper and more extended.
- Dec. 29th. Animal died in severe tetany. Autopsy revealed many extensive suppurating sinuses in the legs, stomach greatly dilated and filled with turbid fluid (no free acid). No trace of thyroid or parathyroid tissue was found.

DISCUSSION

The experiments described above demonstrate that there is a very close similarity if not identity between experimental tetany in animals and various types of clinical tetany in man. The exciting factors which were found to precipitate tetany in the animals that had survived total parathyroidectomy are definitely considered to have an etiological relation to tetany in man. This fact suggests that in clinical tetany there is present an underlying parathyroid deficiency which makes the individual susceptible to diverse intoxications.

Erdheim, Yanase and Haberfelt, have correlated certain types of tetany in man with various anatomical lesions in the parathyroids. Many others (Verebely, Koeingstein, Thompson, Petersen and Thiemich) have failed to find such evidence. The absence of destructive lesions in the parathyroids in tetany cases cannot be interpreted to mean that these glands are not involved in such syndromes. It is quite possible that there may be a physiological glandular deficiency without concomitant anatomical change, a situation seen in the functional achylia. An overwhelming amount of toxic materials in the

circulation, beyond the capacity of the parathyroid tissue to care for them, could easily result in intoxication and yet the glands show no lesions. Destructive lesions in the glands, when coupled with increased absorption of toxic products into the blood (as in intestinal putrefaction in children, intestinal stasis or obstruction, acute dilatation of the stomach or duodenum, acute infections, pregnancy, lactation and other toxemias should produce very severe symptoms. On the other hand, it could be easily possible for extensive pathological changes, amounting to almost an entire destruction of all the parathyroid, to exist without the presence of symptoms of tetany or depression provided the absorption of toxic materials into the blood had been checked by such carbohydrate diets as inhibit intestinal putrefaction and in the absence of the other factors, which we have demonstrated, bear an exciting relation to tetany. It is not difficult to see why pathologists have been reluctant to associate the parathyroids with clinical tetany.

The fact that animals can exist in apparently normal condition with no evidence of parathyroid tissue makes it obvious that the demonstration of pathological alteration in the glands at autopsy in man is not alone conclusive evidence of tetany during life. Much could depend upon the rate of such destruction and the degree of intestinal toxemia. Conversely, the absence of anatomic evidence of injury to the glands in cases of tetany cannot be construed as definite evidence that relative parathyroid deficiency is not responsible for the tetany. In any case tetany must be looked upon as a pathological condition which arises through the accumulation of toxic substances in the blood stream beyond the capacity for the physiologic detoxicating mechanism to care for them.

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A STUDY OF THE SIMULTANEOUS CHANGES IN BLOOD PRESSURE IN THE CAROTID ARTERY AND JUGULAR AND PORTAL VEINS IN ANAPHYLACTIC AND PEPTONE SHOCK IN THE DOG¹

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Received for publication May 18, 1923

The physiologic reactions in anaphylactic and peptone shock in the dog are, as pointed out in previous papers (1), a precipitate fall in arterial blood pressure to 80, 60 or even to 30 mm. Hg; a simultaneous fall in pressure in the proximal end of the jugular vein; a sharp rise in pressure in the portal vein (proximal end of the splenic vein); an increased flow of lymph from the thoracic duct (2); swelling of the liver due to the accumulation of blood in that organ (3), (4); and, after about an hour, hyperemia and even hemorrhage in the intestinal mucosa (3). It was also noted that during shock there was in many dogs a very marked rise in arterial pressure on applying a stimulus (nicotin) which caused labored breathing, but not when this same stimulus did not induce hyperpnea (5).

In searching for the fundamental physiologic reaction in anaphylactic and peptone shock in the dog it is necessary to find a mechanism which will explain all of the above manifestations, especially the rise in pressure in the portal vein, the accumulation of blood in the liver with its consequent enormous increase in size, and the augmented flow of lymph from the liver. It seems hardly possible to explain these changes except as the result of an obstruction to the flow of blood through the liver.

Starling (2) found that the formation of lymph depends upon capillary pressure and the permeability of the capillary walls. In view of the very marked hyperemia of the liver and the increased flow of lymph from this organ, on *a priori* grounds, one would expect the obstruction to be located in the hepatic vein. Shultz (6) demonstrated that sensi-

¹ Aided by a grant from the Fenger Memorial Fund.

tized smooth muscle reacted by contraction to the homologous serum and that normal unstriated muscle responded in a similar manner to peptone. Auer and Lewis (7) showed that anaphylactic shock in the guinea pig was due to the constriction of the smaller bronchioles which, in this animal, are composed of huge masses of smooth muscle.

These facts led to a study of the hepatic vein in the dog by means of Mallory's stain to determine the amount of unstriated muscle which it contained. This vessel in this animal was found to be exceedingly rich in non-striped muscle; it contains relatively enormously greater quantities than does the portal vein. A study of the comparative histology of the hepatic vein has been carried out with Dr. L. B. Arey, the results of which will appear later. It is sufficient here to state that the hepatic vein of the dog was found to be richer in smooth muscle than any of the other twenty-two species studied (8).

The dog, therefore, possesses in its hepatic vein the same type of histologic peculiarity found in the bronchioles of the guinea pig, namely, a relatively huge mass of smooth muscle strategically located so that its contraction will interfere with the outflow of blood from one of the largest and most important vascular areas of the body. An obstruction in the liver due to the contraction of this vein will lead to the impounding of a very large percentage of the total blood of the body in the liver and the splanchnic area in general, in just the same manner as the contraction of the smooth muscle in the bronchioles of the guinea pig prevents the egress of air from the lungs and causes the marked emphysema of the lungs so characteristic of anaphylactic shock in that animal.

Weil (8a) was convinced that anaphylactic shock is a cellular reaction and not a humoral one, for he was unable to demonstrate the presence of any toxic substance in the blood of a dog in the state of anaphylactic shock. He was further convinced that the site of the reaction in the dog is in the liver, just as the seat of the reaction in the guinea pig is in the lungs (3). Weil, however, believed that the parenchyma cells of the liver were the elements which were the site of the reaction. He remarks, "In the guinea pig the chief site of the cellular reaction is smooth muscle tissue. The fact that in both species the fundamental mechanism is a cellular reaction, even though different tissues are involved, furnishes a basis for a uniform theory of anaphylaxis." Weil apparently was not familiar with the presence of the histologic anomaly in the enormous amount of smooth muscle in the hepatic veins of the dog. If it can be proved that the contraction of this smooth muscle

of the hepatic veins at least has a part in the causation of the fall in blood pressure and the increase in size and blood content of the liver in anaphylactic shock in the dog, we have the basis for a still more "uniform theory of anaphylaxis," in that the same tissue will be involved in different animals. The facts that we now have at hand do not furnish absolute proof of this, but they do supply justification for the tentative acceptance of this simple explanation of a remarkable physiologic reaction. The proof of constriction of the hepatic veins as the fundamental reaction in anaphylactic shock in the dog is, after all, on almost as firm a basis as the proof that anaphylactic shock in the guinea pig is due to constriction of the bronchioles, a view which has been quite generally accepted.

As a working hypothesis we have formulated our conception of anaphylactic and peptone shock as follows: The fundamental reaction in these conditions is a cellular one, limited largely if not entirely to non-striated muscle. The particular type of manifestation in different animals depends upon the location of a large mass of smooth muscle in some strategic place where its contraction interferes with some special physiologic function. In the guinea pig, the bronchioles contain large masses of smooth muscle, as shown by Auer and Lewis (7), and its contraction interferes with respiration. Coca (9) found increased resistance to the passage of perfused fluids through the lungs of rabbits during anaphylactic shock; and histologic study showed the pulmonary arteries of the rabbit to present a remarkable degree of muscular development, analogous to that in the bronchioles of the guinea pig. Simonds and Arey (8) found huge quantities of non-striated muscle in the walls of the hepatic veins of the dog. Contraction of this strategically placed smooth muscle in the hepatic vein must be considered in any attempt to explain the manifestations of anaphylactic and peptone shock in the dog, if we are to accept the view that masses of smooth muscle located in the lungs account for the symptoms of this type of shock in the rabbit and guinea pig. The histologic basis for this view is quite as distinctive in the dog as in the guinea pig and rabbit.

Although this conception fulfils the chief demand of a theory, namely, a satisfactory explanation of observed phenomena, we have tried to determine by experiment whether this theory can be placed on the level of established fact. We have, therefore, been carrying out experiments to discover *a*, what influence, if any, the large mass of smooth muscle in the walls of the hepatic veins of the dog has on the fall in arterial pressure in anaphylactic and peptone shock; and *b*, what other factors, if any, may be concerned in this reaction.

In this paper the time relations between the changes in the pressures in the carotid artery and in the jugular and portal veins taken simultaneously, are considered. The pressure in the jugular vein was taken by means of a long, wide cannula in its proximal end, and is therefore practically equivalent to the pressure in the large venous trunks and in the right auricle. This pressure, therefore, furnishes an indication of the degree of filling of the right side of the heart during anaphylactic and peptone shock. The portal pressure was taken by means of a large cannula in the proximal end of the splenic vein.

Two methods have been employed in these experiments. In one series simultaneous blood pressure tracings were taken from all three vessels on smoked drums (fig. 1). The arterial cannula was connected in the usual way with an ordinary mercury manometer, and the pressure measured in millimeters of mercury. The manometers connected with the cannulas in the veins contained a solution of calcium chloride with a specific gravity just one-tenth that of mercury, namely, 1.36. A large glass bulb with two outlet tubes at different levels was connected by a short rubber tube to the cannula in the vein. This bulb was filled to the level of the lower outlet tube with a 2 per cent solution of sodium citrate in 0.9 per cent sodium chloride. The cannula and short rubber tube were filled with the same solution and connected with the lower tube of the bulb. The bulb was then clamped in place in such a way that the level of its contained fluid was the same as that of the cannula in the vein. The second or higher outlet tube from the glass bulb was connected by air-tight rubber tubing with the manometer. A T-tube was inserted in this connection in order to adjust the pressure in the apparatus at the beginning of the experiment. On account of the size of the glass bulb, the level of the fluid in it never varied more than 1 or 2 mm. when the liquid ran into the bulb from the cannula or out of the bulb into the cannula. The manometers recording the venous pressures carried a recording apparatus made of very light materials.

By the second method the arterial pressure was recorded with a mercury manometer in the usual manner. The venous pressures were read off directly at 15-second intervals from a column of liquid (2 per cent sodium citrate in 0.9 per cent sodium chloride) placed against a scale graduated in millimeters. The time was recorded on the kymograph with the arterial pressure by means of an electric marker. All three pressures, at the end of the experiment, were platted on graph paper (figs. 2, 3 and 4).

The blood pressure changes determined by both of these methods showed the same variations in both anaphylactic and peptone shock. In other words, in the dog, so far as blood pressure changes are concerned (carotid artery, jugular and portal veins), anaphylactic and peptone shock are identical. Weil (3) was also convinced of the essential identity of the two reactions.

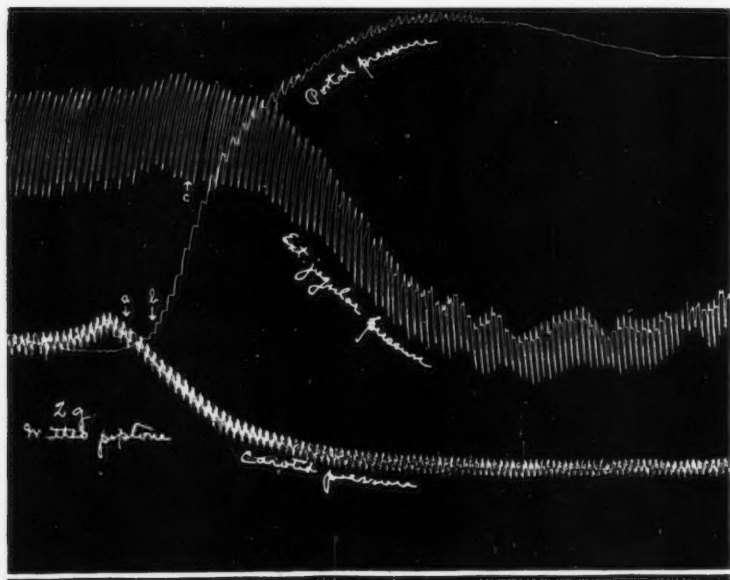


Fig. 1. Direct blood pressure tracings from a fatal case of peptone shock in a dog; *a*, *b* and *c* represent corresponding positions on the several tracings. The rise in portal pressure definitely precedes the fall in arterial pressure. Two grams of Witte's peptone in 10 per cent solution injected into femoral vein.

Blood pressure curves from four dogs are presented in the accompanying charts. All of these animals were of approximately the same size, ranging from 20 to 25 pounds in weight. Two of these dogs were given non-fatal doses of peptone (figs. 3 and 4), and two received doses which proved fatal (figs. 1 and 2). In the "fatal" cases the blood pressure in the carotid artery showed no tendency to rise and the animal died in from 5 minutes to one hour. In the "non-fatal" cases, the arterial pressure rose more or less rapidly to normal.

There appears to be a considerable difference in the susceptibility of different dogs to peptone. The two animals which recovered from the shock received 1.5 and 3 grams of peptone (in 10 per cent solution) respectively; the two animals that succumbed were given 1.5 and 2 grams. All injections were given rapidly into the femoral vein. The same difference in susceptibility has also been noted in anaphylactic

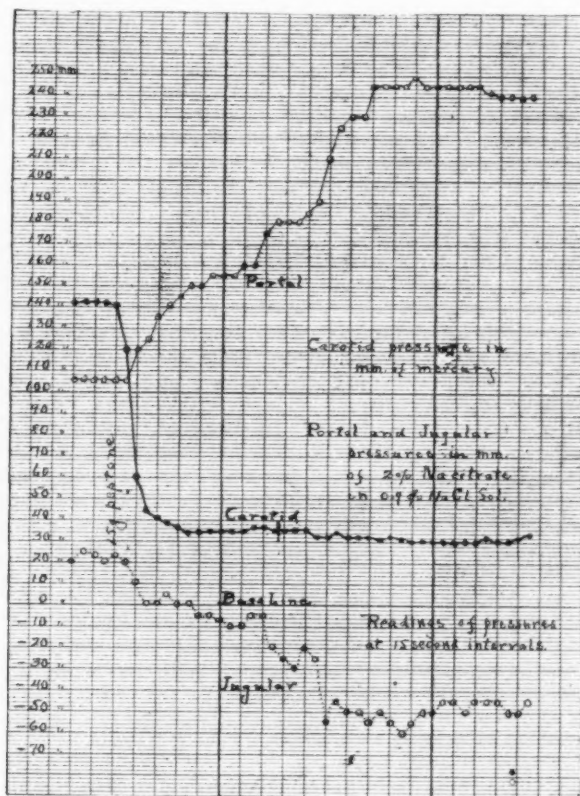


Fig. 2. Portal, carotid and jugular blood pressures in a case of fatal anaphylactic shock in a dog. Readings of pressure levels made at 15-second intervals. Carotid pressure in millimeters of mercury, portal and jugular pressure in millimeters of 2 per cent sodium citrate in 0.9 per cent sodium chloride. One and five-tenths grams of peptone (P. D. & Co.) given, as in figure 1.

shock. Several animals have died after receiving 3 cc. of the serum to which they had been sensitized, while other dogs of approximately the same size have recovered quickly from 5 cc. Different brands of peptone differ markedly in their power to produce shock. Uniform results were obtained in these experiments with Witte's peptone and

with an old peptone sent out as a sample several years ago by Parke, Davis & Co.

The most striking feature of the charts herewith presented is the isochronicity of the changes in pressure in the vessels studied. In the non-fatal cases the arterial and jugular pressures fall and rise together, and the portal pressure moves simultaneously in the opposite direction, namely, it rises while the others are falling and falls while they are rising, and at approximately the same rate. These three pressures return to their previous levels at almost the same time. In the fatal cases, the arterial and jugular pressures fall together and remain low or even continue to fall slowly; while the portal pressure rises and remains at a high level until death. When death intervenes, or while it is in progress, the jugular pressure rises and the portal pressure sometimes drops somewhat. This is in keeping with the work of Bayliss and Starling (10) who called attention to the equalizing of pressures throughout the circulatory system at death,

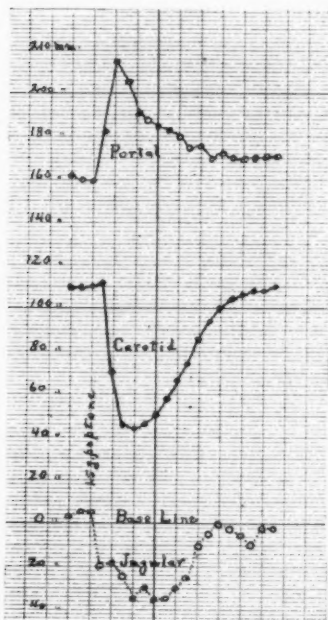


Fig. 3. Portal, carotid and jugular blood pressure tracings in a non-fatal case of peptone shock in a dog. One and five-tenths grams of peptone (P. D. & Co.). Injection and readings as in figure 2.

the blood accumulating in the veins and causing a rise in general venous pressure.

A further study of these charts reveals the fact that in the non-fatal cases pressure changes are evident in the portal and jugular veins 15 seconds after the injection of the peptone into the femoral vein, but the fall in arterial pressure comes distinctly later, after about one-half

minute. Furthermore, the portal pressure begins to fall before the arterial pressure commences to rise. This can be readily seen in figures 3 and 4. It is also shown in table 1, based on the observed pressures during the first two minutes after the injection of peptone.

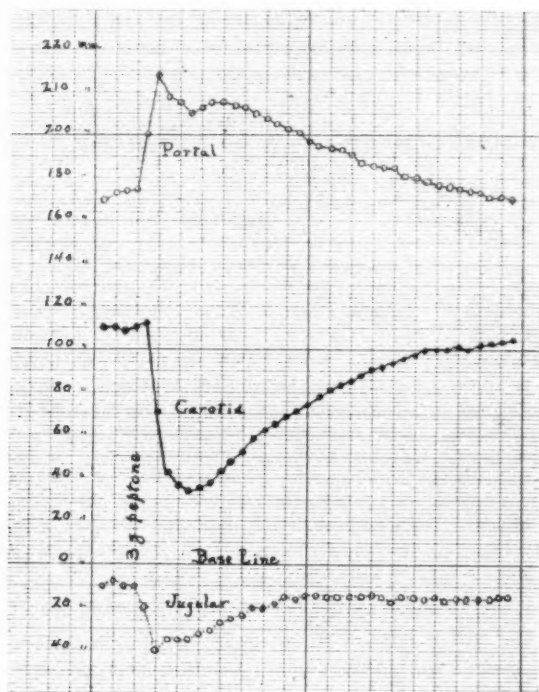


Fig. 4. Portal, carotid and jugular pressures in non-fatal case of peptone shock in a dog. Three grams of peptone (P. D. & Co.). Injections and readings as in figure 2.

In the fatal case (fig. 2) the rise in portal pressure was more gradual; and in this dog the fall in arterial pressure preceded somewhat the rise in pressure in the portal vein. This, however, is not a uniform finding in fatal peptone shock, for many other animals that died from acute shock showed a relation between the pressures similar to that in the non-fatal cases.

The pressure in the proximal end of the jugular vein as taken in these experiments is practically equivalent to the pressure in the right auricle. The rapid and pronounced fall in this pressure indicates that in anaphylactic and peptone shock in the dog the right side of the heart receives less than its normal supply of blood. With its intake thus diminished, the right heart is unable to deliver to the left side of the heart its usual quantity of blood. This is at least one factor in the low arterial pressure in this condition. The increase in the size of the liver and the rela-

TABLE I

Showing the changes from the previous pressures during the first two minutes after injection of peptone

TIME AFTER INJECTION OF PEPTONE		MILLIMETERS ABOVE OR BELOW LEVEL OF PRESSURE PREVIOUS TO INJECTION OF PEPTONE		
		Portal	Jugular	Carotid
Fig. 3	$\frac{1}{4}$ minute	22 mm. above	23 mm. below	2 mm. above
	$\frac{1}{2}$ minute	55 mm. above	21 mm. below	40 mm. below
	$\frac{3}{4}$ minute	45 mm. above	30 mm. below	65 mm. below
	1 minute	30 mm. above	40 mm. below	65 mm. below
	$1\frac{1}{4}$ minutes	28 mm. above	35 mm. below	63 mm. below
	$1\frac{1}{2}$ minutes	25 mm. above	40 mm. below	60 mm. below
	$1\frac{3}{4}$ minutes	23 mm. above	39 mm. below	52 mm. below
	2 minutes	20 mm. above	35 mm. below	42 mm. below
Fig. 4	$\frac{1}{4}$ minute	25 mm. above	10 mm. below	2 mm. above
	$\frac{1}{2}$ minute	54 mm. above	30 mm. below	40 mm. below
	$\frac{3}{4}$ minute	44 mm. above	25 mm. below	68 mm. below
	1 minute	42 mm. above	25 mm. below	74 mm. below
	$1\frac{1}{4}$ minutes	35 mm. above	25 mm. below	76 mm. below
	$1\frac{1}{2}$ minutes	37 mm. above	22 mm. below	75 mm. below
	$1\frac{3}{4}$ minutes	40 mm. above	21 mm. below	72 mm. below
	2 minutes	40 mm. above	18 mm. below	67 mm. below

tively great rise in portal pressure, occurring at the time of the initial fall in jugular pressure and antedating the drop in arterial pressure, indicates that at least a part of the blood which fails to reach the right side of the heart in anaphylactic and peptone shock in the dog is impounded behind some obstruction in the liver.

Three factors may be involved in the fall in portal pressure which precedes the rise in arterial pressure. In the first place, the channels of collateral circulation may be called into play so that the blood in the splanchnic area may be shunted into the general circulation by routes

other than the portal vein. As a general rule, however, collateral circulations are not capable of meeting effectively sudden demands made upon them. In the second place, the obstruction in the liver, which we believe to be due to a constriction of the hepatic veins, may be partially relieved so that more blood is permitted to escape from the liver into the inferior vena cava. That this may be the case is further suggested by the fact that the pressure in the proximal end of the jugular vein begins to rise about the time or a little before the beginning of the upward trend in the pressure in the carotid artery. Finally, the failure of the splanchnic area to receive its usual quota of blood from the depleted arterial supply may render it impossible for the portal pressure to be maintained at its first high level. That this is not the cause of this phenomenon is indicated by the continued high portal pressure in fatal cases.

Inasmuch as the changes in the pressures in these three vessels are so remarkably isochronous and are quantitatively related, it would seem logical to suppose either that some potent factor acts as a primary cause of the change in one of these pressures and that the simultaneous alteration in the other two is secondary to this cause; or that the changes in all three vessels are the effect of some force not directly associated with any one of the vessels in question.

There are three causes of low arterial blood pressure, namely, diminished heart action, dilatation of the peripheral vessels, and an obstruction at some strategic point in the circulation that is capable of preventing the delivery to the left ventricle of the heart of sufficient blood to maintain the systemic pressure at its normal level.

A low arterial pressure due to diminished heart action is associated with an accumulation of blood in the veins with a rise in the pressure in these vessels. Hence it does not appear possible that weakened heart action can account for all of the blood pressure changes observed in anaphylactic and peptone shock in the dog. Auer and Robinson (11) did, however, find electrocardiographic evidence of "disturbances in the conduction of the heart impulses, abnormalities in ventricular contractions, and other unusual disturbances in the mechanism of the heart beat" in anaphylactic shock in the dog.

In several dogs lowered arterial pressure was induced by the intravenous injection of nitroglycerine and by inhalation of amyl nitrite. There was a simultaneous fall in the pressures in the carotid artery and in the jugular vein with either no change or a very slight fall in portal pressure. The change in venous pressure was proportionally less than

that in the artery. A consideration of the mechanics of the circulation as well as the experimental evidence just cited makes it apparent that peripheral dilatation alone cannot account for all of the changes in blood pressure observed in anaphylactic and peptone shock.

Except for failure of the right side of the heart itself, there appear to be only two strategic points in the circulation in which obstruction can prevent the delivery to the left side of the heart of sufficient blood to maintain the systemic pressure at its normal level, namely, in the lungs and liver. In both of these locations the obstruction must occur suddenly; if it develops gradually, the circulatory system may be able to adapt itself to the changed conditions and no marked alterations in pressure result. The fact that a sudden and marked obstruction in the pulmonary circulation may cause a fall in arterial pressure is self-evident.

Evidence that the liver occupies a similar strategic position in the circulatory system is supplied by the known facts concerning the quantity of blood in the liver and the capacity of the liver and splanchnic area in general for impounding blood. Burton-Opitz (12) estimated that the equivalent of the total quantity of blood in the body passed through the liver every three minutes. Macleod and Pearce (13) found that the total outflow of blood from the liver varies in the dog between 1.06 and 2.40 cc. per second per 100 grams of liver. Stewart (14) states that the liver contains "rather more than one-fourth" of the total blood content of the body. Weil (3) estimated that the amount of blood in the liver of a dog in anaphylactic shock was the equivalent of 61 per cent of the total quantity of blood in the body. If in addition to this, the storage capacity of the vessels of the gastro-intestinal tract is taken into consideration, it is evident that practically the entire quantity of blood in the body of a dog can be impounded behind the hepatic veins.

That the liver of the dog becomes enormously distended with blood in anaphylactic and peptone shock has been shown by my own experiments and those of Edmunds (4), Weil (3) and others. Weil (14a) produced a localized area of very marked hyperemia of the liver by injecting beneath its capsule a minute amount of the serum to which the dog had been sensitized. Injection of the antigenic substance into one branch of the portal vein produced an immediate severe congestion of the corresponding lobe of the liver. Comparable results were obtained by Weil from the injection of peptone in a similar manner into the livers of normal dogs. I have found that a very violent reaction follows the

injection into a branch of the mesenteric vein of a very small amount of a serum to which the dog had been sensitized. From 50 to 100 mgm. of peptone, similarly injected, caused symptoms of profound shock in a normal dog.

The gross and microscopic findings in a dog dead of very acute anaphylactic and peptone shock are practically limited to the liver, as pointed out by Weil (3) and confirmed by my own experiments. A study of the gross and microscopic findings will be presented in a later paper.

Erlanger and Gasser (15) "have presented evidence indicating that the accumulation of blood in the portal area as a result of increased portal-hepatic resistance is not the cause of the failure of the circulation (after injection of adrenalin for a period of 20 to 30 minutes). For marked obstruction of the hepatic radicles in the liver by the injection of a suspension of lycopodium spores may not lead to the shock-like failure of the circulation seen after adrenalin." The lycopodium spores injected into the portal vein "plug the vessels proximal to the point where they break up into capillaries." It is evident that the obstruction to the flow of blood through the liver as a result of the injection of lycopodium spores is quite different from that which would result from a constriction of the branches of the hepatic vein. In the first place, the impounding capacity of the liver itself would be excluded very largely by the location of the mechanical obstruction due to the spores. Furthermore, obstruction in the hepatic veins would exert its impounding action on the blood reaching the liver by both portal vein and hepatic artery; while the obstruction due to the spores would affect only that from the portal vein. Macleod and Pearce (13) found that occluding the hepatic artery reduced the outflow of blood from the liver by about 30 per cent. Burton-Opitz (17) estimated that the proportion between hepatic artery blood and portal vein blood in the liver of the dog was as 25 to 59. Hence approximately 30 per cent of the blood reaching the liver under normal conditions comes through the hepatic artery. This supply would not be interfered with by the plugging of the smaller venous radicles proximal to the point where they break up into capillaries, unless it resulted from the lowered general arterial blood pressure. Burton-Opitz (17) demonstrated that obstruction of the portal vein may cause a fall in pressures in the femoral and hepatic arteries, and a reduction of the amount of blood passing through the latter vessel. "But if there was some connection between the portal vein and the vena cava to eliminate stagnation of blood in the branches

of the portal vein when it was obstructed, there was no fall in pressure in either of the arteries." If, as this work of Burton-Opitz indicates, obstruction of the portal vein alone can cause a fall in arterial blood pressure, an obstruction so located that it affects the total blood flow into the liver would be even more effective in inducing a lowering of pressure in the arteries.

The conditions present in anaphylactic and peptone shock in the dog cannot be judged by the changes in blood pressure induced by long-continued injection of adrenalin. It is possible, as suggested by Lamson (18), that adrenalin may exert its effect on the hepatic vein of the dog. The vasoconstriction of the vessels in the gastro-intestinal tract, however, reduces the amount of blood reaching the liver to such an extent that impounding of blood in this organ and in the splanchnic area in general could not be as marked as in anaphylactic shock because of the diminished inflow. These interesting experiments of Erlanger and Gasser cannot, therefore, be considered as a basis of argument against the conception of anaphylactic and peptone shock set forth in this paper.

Manwaring (19), while admitting that constriction of the hepatic veins will "readily account for the hepatic engorgement, splanchnic congestion and low systemic pressure" objects to this view on the ground that this "hypothesis would necessitate the assumption that the hepatic blood vessels acquire properties during sensitization that are not acquired by other tissues." This objection is equally valid against the now generally accepted conception of the mechanism of anaphylactic shock in the guinea pig.

That the blood vessels of different organs may react quite differently to the same reagent has been demonstrated by numerous observers. Langley (20), Brodie and Dixon (21), Cow (22) and others have shown that adrenalin causes vasoconstriction of most blood vessels, but causes a dilatation of some vessels. Barbour and Prince (23) found a difference in the reaction of the same vessel in different animals to adrenalin. According to Brodie and Dixon (21), the action of adrenalin upon any tissue is invariably that which follows excitation of the sympathetic nerves supplying the tissue. Its action on blood vessels is proportional to the innervation of the vessel in question. Barbour (24) also found that the energy of the response of a blood vessel to adrenalin was proportional to the quantity of smooth muscle in its walls. Hoskins and Gunning (25) demonstrated that adrenalin causes a constriction of the skin of the leg but a marked dilatation of the vessels of the underlying skeletal muscles.

Hence difference in the behavior of different vessels of the body in anaphylactic and peptone shock may actually occur without its being an isolated and peculiar example of such a condition. The alleged necessity of assuming "that the hepatic blood vessels acquire properties during sensitization that are not acquired by other tissues" is not, therefore, a competent and adequate objection to the conception of the physiologic mechanism of anaphylactic and peptone shock in the dog as here set forth.

SUMMARY

An analysis of the manifestations of anaphylactic and peptone shock in the dog strongly suggests an obstruction to the flow of blood through the liver, the obstruction being so located as to become effective after the blood had passed through the sinusoids. A study of the hepatic vein in the dog reveals the fact that it contains a relatively huge mass of smooth muscle. This smooth muscle occupies a strategic position with reference to the circulation of blood not unlike that which the very large mass of non-striated muscle in the walls of the bronchioles of the guinea pig occupies to the circulation of air in the lungs. It has been shown by many other observers that smooth muscle is very sensitive to peptone and to any foreign proteid to which the animal has been sensitized. It is a very generally accepted view that anaphylactic shock in the guinea pig is due to the contraction of the mass of smooth muscle in the walls of its bronchioles. It appeared possible that the manifestations of anaphylactic and peptone shock in the dog might be the results of contraction of the mass of non-striated muscle in the hepatic vein. Experiments have therefore been carried out to determine what part, if any, constriction of the hepatic veins may play in anaphylactic and peptone shock in the dog. In this paper the results of a study of blood pressure changes taken simultaneously in the carotid artery and jugular and portal veins, are recorded. These experiments show:

1. There is a striking isochronicity in the pressure changes occurring in these three vessels.

2. The arterial and jugular pressures fall rapidly and simultaneously. The pressure in the jugular vein, as taken in these experiments, is equivalent to the pressure in the right auricle. The changes in these two pressures indicate, therefore, *a*, that the right side of the heart is receiving less than its wonted quota of blood; and *b*, that the fall in arterial pressure is in part at least due to the inability of the right heart to deliver to the left side sufficient blood to maintain systemic pressure at its normal level. This is further indicated by the fact that these two pressures rise simultaneously during recovery.

3. During this type of shock in the dog there is a sharp rise in the pressure in the portal vein taken from the proximal end of the splenic vein. An examination of the time relations existing among these three pressures shows that the rise in portal pressure precedes the fall in arterial and jugular pressures, and that the portal pressure begins to fall before there is a definite rise in carotid and jugular pressures.

4. Evidence is adduced that constriction of the hepatic veins can satisfactorily account for these pressure changes. In other words, the experiments here described tend to confirm the view that the fundamental mechanism of anaphylactic and peptone shock in the dog has its physiologic basis in a constriction of the hepatic veins and its anatomic basis in the presence of a histologic peculiarity in the form of a relatively huge mass of smooth muscle in the walls of these vessels.

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THE EFFECT OF HIGH AND LOW TEMPERATURES ON THE CATALASE CONTENT OF PARAMECIUM AND SPIROGYRA

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Received for publication May 21, 1923

Barratt (1) showed that the carbon dioxide elimination of paramecium caudatum was decreased by low temperatures and increased by temperatures higher than the normal. Leichsenring (2) finds that oxygen consumption of paramecia is also decreased by low temperatures and increased by temperatures higher than the normal. Hence, the respiratory metabolism of these organisms is decreased by lowering the temperature and increased by raising it.

The object of the following experiments on paramecia caudata was to determine whether low temperatures would produce a decrease and temperatures higher than the normal an increase in their catalase content. These organisms were grown on an infusion made of dried leaves of the pond lily and tap water, at a temperature of approximately 20°C. Five hundredths cubic centimeter of the organisms was added to 15 cc. of neutral hydrogen peroxide in a bottle and the amount of oxygen liberated in 20 minutes was taken as a measure of the catalase content. The measurement of the 0.05 cc. gave some trouble and for that reason a detailed description of the procedure is given here. The measure was made by sealing off one end of a 10 cm. piece of an ordinary 1 cc. glass pipette graduated to hundredths of a cubic centimeter. After collecting, centrifugalizing and washing the paramecia with aerated water, they were introduced into the measure. The measure was then placed in a small electric centrifuge making about 1200 revolutions per minute and centrifugalized for approximately a minute and a half. The debris which collects on the surface and the excess organisms were drawn off by means of a fine pointed pipette. The 0.05 cc. of the organisms left in the measure could then be transferred and used as desired.

A large quantity of the organisms was collected and the catalase content of 0.05 cc. of these determined. The liquid containing the organisms was then divided into three parts of approximately 100 cc. each and introduced into flasks. One of the flasks was kept in a bath at 0°C . for three hours, one in a bath at 32°C . for a similar length of time and the remaining flasks kept at ordinary room temperature, 22°C . At the end of the three-hour period catalase determinations were made and it may be seen in figure 1 that the catalase of the paramecia kept at 32°C . had been increased 5 per cent and that the catalase of those kept at 0°C . had been decreased 32 per cent. At the end of the three

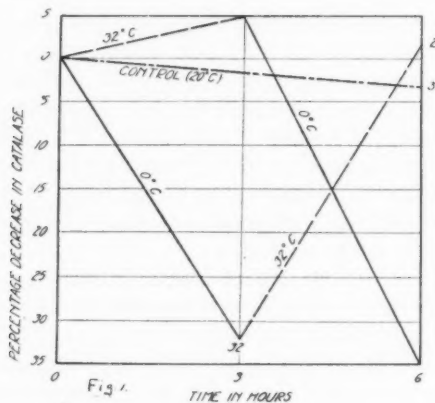


Fig. 1. Curves showing the decrease in the catalase content of paramecium caudatum brought about by lowering the temperature to 0°C . and the increase in catalase brought about by raising the temperature to 32°C .

hours the paramecia in the 32°C . bath were transferred to the 0°C . bath and those in the 0°C . bath to the 32°C . bath. Three hours later catalase determinations were made again and in figure 1 it may be seen that the effect of raising the temperature of the paramecia which had been kept for three hours at 0°C . to 32°C . for three hours was to increase greatly the catalase content and that the lowering of the temperature of those that had been kept for three hours at 32°C . to 0°C . for three hours resulted in a great decrease in catalase. It may be seen further that there had been little change in the catalase content of the control organisms kept at ordinary room temperature. Several experiments similar to the preceding were carried out with comparable results. In

some of the experiments the increase in catalase produced by 32°C. was greater than that shown in figure 1.

A second large quantity of paramecia was collected and the catalase content as well as the oxygen consumption of 0.05 cc. of these organisms determined. The liquid containing the organisms was then divided into four parts of approximately 100 cc. each and introduced into flasks. Two of the flasks were placed in a bath at 0°C. while the two remaining flasks were kept in the room at the ordinary temperature, 20°C. At the end of three hours 0.05 cc. of the organisms was removed and determinations of the oxygen consumption and catalase

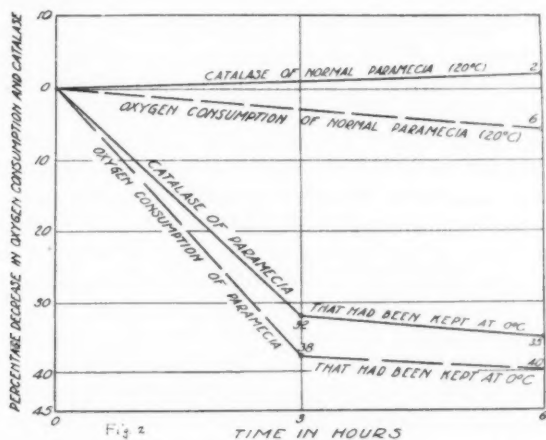


Fig. 2. Curves showing the decrease in the catalase content and oxygen consumption of paramecia brought about by lowering the temperature to 0°C.

content made. At the end of six hours similar determinations were made of the organisms kept at 0°C. and at ordinary room temperature. The results of these determinations are shown in figure 2. It may be seen that there was little change in the catalase content or the oxygen consumption of the organisms that had been kept at ordinary room temperature, but that both the catalase and oxygen consumption were decreased in the organisms kept at 0°C. The decrease in catalase after three hours was 32 per cent and in oxygen consumption 38 per cent and after six hours the decrease in catalase was 35 per cent and in oxygen consumption 40 per cent. The oxygen consumption of the organisms was determined according to the Rideal-Stewart (3) modification of the

Winkler method. Five hundredths of a cubic centimeter of the organisms and 35 cc. of aerated water were used in making the determinations. Several experiments similar to the preceding were carried out with comparable results.

We (4) had found that low temperatures increased the blood catalase of warm-blooded animals and decreased it in a cold-blooded animal in keeping with the fact that low temperatures increase oxidation in warm-blooded animals and decrease it in cold-blooded animals. The following experiments were carried out to determine if low temperatures would affect the catalase content of the red blood cells of a warm- and of a cold-blooded animal, as they do the unicellular organism, paramecium. The warm-blooded animal used was the cat, and the cold-blooded animal, the turtle. It may be recalled that the catalase of the blood is found almost exclusively in the red blood cells and, furthermore, that the erythrocytes of the cold-blooded animal contain a nucleus, while those of the warm-blooded animal do not. Twenty cubic centimeters of cat's blood and 10 cc. of turtle's blood were collected and defibrinated. The catalase content of these two samples of blood was determined by adding 0.3 cc. of the cat's blood to 150 cc. of neutral hydrogen peroxide and 1.0 cc. of the turtle's blood to 100 cc. of peroxide and the amount of oxygen liberated in ten minutes was determined. The two samples of blood were then divided into two parts. One portion of each sample was placed in a bath at 0°C. and the other kept at 20°C. Catalase determinations were made after 3, 5 and 7 hours. It may be seen in figure 3 that low temperatures had no effect on the catalase content of the blood in vitro of either the warm-blooded or the cold-blooded animal.

As the result of the work of Ingen-House (5), Senebier (6) and De Saussure (7) on plants, it is known that animals and plants alike in their respiratory processes take up oxygen from the air and give off carbon dioxide. The most effective way of increasing the respiratory metabolism in plants is by raising the temperature and of decreasing it by lowering the temperature. Light produces only a slight increase in the respiratory metabolism of plants, although it is essential for photosynthesis (8).

The object of the following experiments was to determine if the catalase content of *Spirogyra porticalis* would be increased by raising the temperature and by light and decreased by lowering the temperature.

We were fortunate in having an almost inexhaustible supply of practically a pure culture of *Spirogyra porticalis* in a nearby lake. The lake

was frozen almost the entire time during the months of January and February, when most of the following experiments were carried out.

The catalase determinations were made as follows. The desired amount of *Spirogyra* was placed in a cloth and the excess water removed by squeezing with the hand. This material was then ground through a small hashing machine twice. One and twenty-five hundredths gram of the ground material were added to 15 cc. of neutral hydrogen peroxide at 18°C. in a bottle and the amount of oxygen liberated in twenty minutes was taken as a measure of the catalase content. The bottles were shaken at a slow rate in a shaking machine during the determinations.

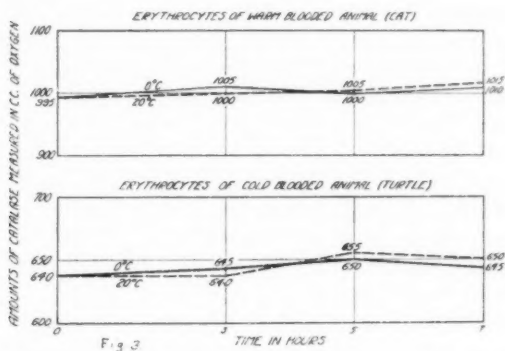


Fig. 3. Curves showing that low temperatures have no effect in vitro on the catalase content of the red blood cells of the warm-blooded or of the cold-blooded animal.

A large amount of *Spirogyra* was removed in the ice-cold water to the warm laboratory (22°C.) and catalase determinations made immediately, and after four and twelve days. The material which was used immediately, liberated 29 cc. of oxygen from hydrogen peroxide in 20 minutes; that kept in the warm laboratory for four days, 52 cc. and that kept twelve days, 62 cc. The *Spirogyra* remaining after the previous determinations were made was taken back to the lake at the end of the twelve days and placed in the ice-cold water in an enclosure made for it. After eight days catalase determinations were made again and 1.25 gram of this material liberated 37 cc. of oxygen from hydrogen peroxide in twenty minutes. It should be mentioned in this connection that the water in which this material was placed was frozen most of the eight days.

A large quantity of *Spirogyra* was collected and brought to the laboratory in ice-cold water. One and twenty-five hundredths gram of this material liberated 31 cc. of oxygen from hydrogen peroxide in twenty minutes. The large batch of *Spirogyra* was divided into four smaller batches. Each of these was placed in ten liters of lake water in a container measuring 40 cm. in diameter. Two of the batches were placed in the dark at 0°C. and 30°C., while the remaining two were kept at the same temperatures and each exposed to a 200 watt light with a frosted bulb at a distance of one meter, for twenty-four hours. At the end of this time determinations were made and it was found that the *Spirogyra* kept at 0°C. in the light liberated 32 cc. of oxygen and that in the dark 33 cc.; that kept in the light at 30°C. liberated 47 cc. of oxygen and that kept in the dark at 30°C. liberated 40 cc. from hydrogen peroxide. By comparing these figures it may be seen that there was no change in the catalase content of the *Spirogyra* kept at 0°C. either in the dark or in the light: that the catalase was increased in the *Spirogyra* kept at 30°C. and the increase was greater in the *Spirogyra* kept in the light than that kept in the dark. It should be mentioned in this connection that Appleman (9) found in the greening and sprouting of potatoes an increase in catalase corresponding with the increase in respiration. Crocker and Harrington (10) found a similar relation between catalase and respiration in the germination of seeds.

In the month of January a large amount of *Spirogyra* was placed in an enclosure in the ice-cold water of the lake. Catalase determinations were made and it was found that 1.25 grams of the materials liberated 33 cc. of oxygen from hydrogen peroxide in twenty minutes. Similar determinations were made the first of May when the temperature of the lake water was 17°C. and 1.25 gram of the material liberated 56 cc. of oxygen from hydrogen peroxide in twenty minutes. A similar comparison of the catalase content of the blood of rabbits in winter and in summer had already been made (11), and we found that the catalase content was lowest in summer when the weather was hottest and highest in winter when the weather was coldest.

SUMMARY

Low temperatures decrease the catalase content of paramecia and temperatures higher than the normal increase it, in keeping with the fact that low temperatures decrease the respiratory metabolism and high temperatures increase it.

Similarly lowering the temperature decreases the catalase content and the metabolism of *Spirogyra* and raising the temperature increases the catalase content and metabolism. Light also produces an increase in the catalase content and respiratory metabolism of *Spirogyra*.

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STUDIES ON THE VISCERAL SENSORY NERVOUS SYSTEM

XV. NOTE ON THE INNERVATION OF THE CARDIA IN THE MACACUS MONKEY

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Received for publication May 21, 1923

In recent reports¹ we have described a new method of recording the contractions of cardia, and using this method on dogs, rats and rabbits, we found that the cardia is supplied with motor as well as with inhibitory nerve fibers both from the vagi and the splanchnic nerves. We also showed that reflexes to the cardia can be induced by the stimulation of any afferent nerve; some of the reflexes pass via the splanchnic nerves; the visceral afferents induce predominantly motor reflexes (spasms) into the cardia.

We have extended these observations to one specimen of *Macacus* monkey, a young female, in good condition, using essentially the same technic as in the observations on dogs and cats. The animal was kept under ether anesthesia, the cardiometer put in place via an incision in the fundus of the stomach and a slit in the esophagus in the neck. The reader is referred to our former reports¹ for details of the technic, but it may be stated here that when the cardia contracts strongly the delicate rubber balloon in the cardia is compressed so that the respiratory changes in intrathoracic pressure are not communicated to the water manometer connected with the stomach end of the cardiometer. Only one monkey was available for these observations, and ordinarily we do not base conclusions on observations on only one animal, but in this case the results are put on record as they are practically identical with the data definitely established for the dog, cat and rabbit.

1. With the cardiometer in place and without any positive pressure in the balloon, the cardia of the monkey exhibited continuous rhythmic contractions. The tonus and contractions of the cardia decreased

¹ Carlson, Boyd and Percy: *This Journal*, 1922, lxi, 14; *Arch. Int. Med.*, 1922, xxx, 409.

gradually during the course of the experiment until after two hours of anesthesia and various experimental stimulations the cardia became atonic and showed no spontaneous rhythm.

2. With the vagi and splanchnic nerves intact the following manipulations caused prolonged contractions of the cardia: mechanical stimulation of the posterior nares, pinching or cutting the skin, traction or pressure on the urinary bladder, pressure on the small and large intestines.

3. Tetanization of the peripheral end of the splanchnic nerves induced prolonged contraction of the cardia. This was confirmed by

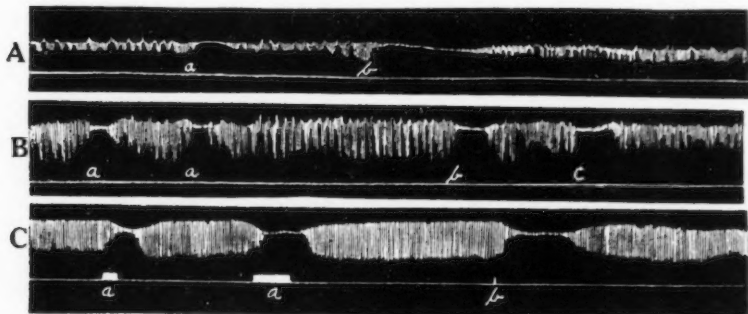


Fig. 1. Monkey. Ether anesthesia. Water manometer tracings from the gastric end of the cardiometer (decreased amplitude of excursions = greater tonus of the cardia). A: *a* = mechanical stimulation of the nares; *h* = incision through the abdominal wall. B: *a* = mechanical stimulation of the urinary bladder; *b* = mechanical stimulation of the small intestines; *c* = mechanical stimulation of the large intestine. C: *a* = tetanization of the peripheral end of the right splanchnic nerve; *b* = intravenous injections of 1 cc. 1-50,000 adrenalin. Showing reflex contraction of the cardia from cutaneous and visceral afferents; motor fibers to the cardia from the splanchnic nerves; and contraction of the cardia from adrenalin.

direct inspection, after opening the chest and maintaining the animal by artificial respiration.

4. Stimulation of the peripheral end of the vagi causes contraction of the cardia.

5. Intravenous injection of dilute solutions of adrenalin (1 cc. 1-50,000) caused prolonged contraction of the cardia.

This experiment shows that the innervation and reflex control of the cardia in the monkey is the same as in the other mammals (dog, cat, rabbit). Motor fibers reach the cardia both via the splanchnic

and the vagi nerves. In the case of the cat, dog and rabbit it was shown that inhibitory fibers reach the cardia via the same nerves. We were unable to settle this point for the monkey.

These observations place the monkey in line with the carnivora and herbivora in its complexity of innervation and reflex control of the cardia and lower end of the esophagus.

STUDIES ON RENAL TUBULE FUNCTION

III. OBSERVATIONS ON THE EXCRETION OF SULPHATE, WITH A MODIFIED TECHNIQUE FOR THE DETERMINATION OF INORGANIC SULPHATE IN BLOOD OR PLASMA.

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Received for publication May 28, 1923

In the first paper (1) of this series the concentration ratios of chloride, urea, phosphate and of sugar in the phlorhizinized dog were compared. The comparisons were made on single samples of plasma and urine, taken at a time when the plasma concentration of the substances under observation, except in the case of sugar in the phlorhizinized dog, had been raised to a high level by previous enrichment of the organism through intravenous injection. It was found that under the conditions of the experiments the concentration ratios of phosphate and of sugar in the phlorhizinized dog were approximately the same, urea being concentrated to a less extent and chloride the least of all. The plan of that set of experiments is seen to be similar to that employed by Mayrs (2), but the interpretation of results was quite different from his. In the second paper (3) an extension of the observations to consecutive periods through which the concentration ratios of various bodies were followed with varying plasma concentration and rate of urine flow demonstrated conclusively that the conception of a pure filtration-reabsorption basis of kidney function, with bodies of equal concentration ratios classed as no-threshold bodies, is incorrect. It was shown that a true secretion of urea, phosphate and of sugar in the phlorhizinized dog must be invoked. This secretion has been assigned to the tubules. It is not proposed to go here into a discussion of the evidence for and against a true glomerular secretion. Evidence offered by many previous investigators in favor of a tubular secretion is so strong and that in favor of a glomerular so scanty that, a true secretion of certain urinary constituents being conceded, the burden of proof must rest on those who would assign this function to the glomeruli.

The three bodies which Mayrs found to have approximately equal concentration ratios and which he therefore classed as no-threshold bodies are creatinine, phosphate and sulphate. The reasons for omitting creatinine from these observations have already been given (1). Since, however, Mayrs drew most of his conclusions from comparisons of other urinary constituents with sulphate, it was felt that sulphate should be included in our observations. It seemed particularly desirable to extend the comparison with sulphate to several consecutive periods of varying plasma concentration and rate of urine flow. When the experiments reported in the preceding papers (1), (3) were performed we could find no technique described for the determination of sulphate in small amounts of blood or plasma. The gravimetric method, with precipitation by BaCl_2 , is not practicable where one must take several samples of blood at short intervals, since the loss of the amount of blood required would seriously disturb the animal's circulation. Denis (4) has described a nephelometric method for the determination of sulphate in small amounts of blood. The method is applicable to normal and pathological bloods, i.e., to bloods showing a retention of sulphate, as in nephritis. Certain technical difficulties attend the use of this method in an experiment where the plasma sulphate concentration has been raised to many times the normal by a previous intravenous injection of sulphate and where consequently the sulphate value cannot be predicted with any great certainty. For a nephelometric reading the standard must agree quite closely with the unknown. In a blood where the sulphate concentration may be anywhere from 20 to 150 times the normal it is obvious that a large series of dilutions and of standards must be made. This objection is not so serious in working with clinical cases, for here, if none of the dilutions happens to agree sufficiently with a standard, it is a simple matter to get another blood sample and, with the knowledge gained from the first trial, to prepare a dilution and standard of proper strength. In experiments such as those reported here, however, a blood sample must be taken once and for all.

We turned next to the principle of precipitation of the sulphate in combination with benzidine. After considerable preliminary work it was decided that a modification of Fiske's technique (5) which would render it applicable to blood seemed most practicable. It was found that after an intravenous injection of 10 cc. per kilo of 5 per cent anhydrous Na_2SO_4 in 20 minutes, the rates of injection employed in the present experiments, the plasma sulphate concen-

tration was such that the determination could be carried out on 2 to 4 cc. of plasma. It was also found that, except in the cases where the plasma phosphate level was greatly increased by intravenous injection of phosphate, the ratio of sulphate to phosphate was high enough that a removal of the phosphate preliminary to precipitation of the sulphate is unnecessary. The method is equally applicable to plasma or whole blood. No attempt was made to determine other than the free inorganic sulphate.

The method consists in the precipitation of protein by trichloroacetic acid, evaporation and ashing of the trichloroacetic filtrate, dissolving the ash and proceeding from this point as in Fiske's sulphate method for urine. As stated above, the removal of the phosphate can be dispensed with in bloods with normal phosphate content. A departure from Fiske's method of filtering off the benzidine sulphate precipitate has also been made.

Technique. Precipitate 1 volume of plasma or citrated blood in 3 volumes of distilled water with 1 volume of 20 per cent trichloroacetic acid and filter. Take 10 to 15 cc. of the filtrate in 100 cc. beaker, add 2 cc. concentrated HNO_3 and evaporate under hood on electric hot plate. Do not boil, as loss by spattering is very likely to occur. When almost dry and before charring has occurred, add 2 cc. more of HNO_3 . This must be repeated several times, usually three or four, depending on the non-protein organic content of the plasma. Finally let the ash dry and if it does not turn darker than a light brown, turn on the current to its full strength and let the beaker sit with the full heat on for ten minutes. If blood has high phosphate content, rinse into a test tube marked at 12 cc., add 1 drop concentrated ammonium hydroxide, 1 cc. 5 per cent ammonium chloride solution, about 0.1 gram finely powdered dry basic magnesium carbonate and make up to mark. Shake one minute and pour into a 7 cm. filter paper enough of the suspension to fill nearly to top, allowing this to drain back into tube. Filter into dry container and take 10 cc. filtrate in 100 cc. beaker. Add 2 drops of methyl orange solution and $\text{N}/1$ HCl until solution is red. Then add 2 cc. benzidine reagent, let stand few minutes, add 4 cc. of 95 per cent acetone and let stand 10 minutes more. The benzidine reagent is prepared according to Fiske's directions by suspending 4 grams of benzidine in about 150 cc. of water in a 250 cc. volumetric flask and adding 50 cc. of standardized $\text{N}/1$ HCl . Shake until dissolved and make up to volume. Filter if necessary.

Prepare filter for separating the precipitate of benzidine sulphate according to following. Put small Gooch crucible in suction flask, place circular piece of filter paper cut to fit on bottom of flask, pour on this a suspension of paper pulp and apply suction. Make mat of paper pulp about 1 mm. thick. On this place another circular piece of filter paper, wet and pack down with large glass rod flattened on one end. Pour contents of beaker slowly down small glass rod into Gooch, applying suction. Rinse out beaker three times with 2 to 3 cc. 50 per cent acetone from a pipette, pouring rinsings on paper mat. Then wash mat and sides of crucible three times with 2 to 3 cc. of 50 per cent acetone. With fine forceps transfer mat to its original beaker, taking care to insert forceps under side of mat so that precipitate is not disturbed and wiping down sides of crucible with bottom of mat. Or, if one wishes, the crucible may be transferred to the beaker along with the mat, to safeguard against loss of precipitate by adhering to side of crucible. Add about 10 cc. of distilled water to mat in beaker, boil a few seconds, stirring with a glass rod. Run in about 1 cc. N/50 Na OH, boil again for a few seconds, continue titration, adding phenol red at this point, until further boiling fails to discharge pink color.

The removal of the phosphate by the magnesium carbonate is not necessary in bloods with normal phosphate content. After removing beaker with ash from hot plate dissolve ash in 8 to 10 cc. water, add 2 drops methyl orange and N/1 HCl until solution is red. Then add benzidine reagent and acetone and proceed as above.

Remarks on method. All reagents and filter paper must obviously be free from sulphate. It is not necessary that the solution on dissolving the ash be absolutely water clear. A light straw color does no harm. The method of filtering off the precipitate with a Gooch and paper mat was suggested by Dr. A. P. Briggs as being simpler than Fiske's special filtration tube. A certified or carefully calibrated burette graduated in twentieths of a cubic centimeter should be used for the titration. Calculate milligrams S per 100 cc. blood or plasma from cubic centimeters of N/50 NaOH required to titrate H_2SO_4 in precipitate.

Results of method. Figures are given on two dog plasmas to which a known amount of K_2SO_4 had been added. The sulphate content of the plasma alone was first determined by Denis' method (4). The sulphate content of these plasmas was so low that it was necessary to employ the technique recommended for human blood. Even with this technique the cloud obtained on one of the plasmas was so slight

that it could not be read in the nephelometer, i.e., there was considerably less than 0.5 mgm. S per 100 cc. The figures given in table 1 were obtained by adding K_2SO_4 equivalent to 0.5 mgm., and 1 mgm. S to 2 cc. samples of plasma. The added S is thus equal to 25 and 50 mgm. S per 100 ccm. plasma.

Experimental procedure. As stated earlier in this paper, the purpose of the present experiments is to compare the concentration ratio of sulphate with that of urea, phosphate and of sugar in the phlorhizinized dog through several consecutive periods with a varying plasma concentration of the bodies followed and a varying rate of urine flow. Analyses of urea, phosphate and sugar were made by methods mentioned in a previous paper (1), plasma sulphate by the technique described above, urine sulphate by Fiske's method (5)

TABLE 1

	MG. S PER 100 CC.			
	Inorganic S in plasma	S added	Theory	Found
Plasma 1.....	Trace	25	25.0	24.7
Plasma 1.....	Trace	50	50.0	50.3
Plasma 2.....	0.5	25	25.5	25.1
Plasma 2.....	0.5	50	50.5	51.4

with described change in filtration technique. Male dogs were used in all experiments. The experimental procedure was much as described in the preceding papers. The animals were given hypodermically 0.032 gram morphine sulphate per 10 kilos body weight, put under ether, a tracheal cannula inserted, a femoral artery exposed for drawing blood and a femoral vein for the injecting burette. The bladder was catheterized and emptied before starting urine collection. Protocols of experiments with tabulated results follow.

Experiment 1. Comparison of sulphate with phosphate. Body weight of dog 9.34 kilos. Intravenous injection of 10 cc. per kilo of 5 per cent anhydrous Na_2SO_4 solution started at 10:25, ended at 10:55. Bladder emptied and urine collection started at 10:55. Intravenous injection of 30 cc. per kilo per hour of 0.9 per cent NaCl solution started at 10:55 to keep up diuresis, changed to 30 cc. per kilo per hour of 1.8 per cent NaCl at 11:25, this continued through urine collection periods. Urine collection made every 15 minutes, blood sample taken in middle of each collection period. Results in table 2.

It is seen that the concentration ratios of S and P are in no period even approximately the same. It will be noted that this experiment departs from Mayrs' plan of procedure in that while the sulphate concentration is raised experimentally, the phosphate concentration is allowed to remain at its normal level.

In the next experiment the plasma concentration of both sulphate and phosphate is increased by the intravenous injection of a mixture of sulphate and phosphate. The intravenous injection of NaCl solution

TABLE 2

SAMPLE	MGM. S PER 100 CC. PLASMA	MGM. S PER 100 CC. URINE	CONCENTRATION RATIO OF S	MGM. P PER 100 CC. PLASMA	MGM. P PER 100 CC. URINE	CONCENTRATION RATIO OF P	URINE, CC. PER HOUR	MGM. S PER HOUR	MGM. P PER HOUR	CONCENTRATION RATIO OF P, S = 100
1	50.0	461	9.2	2.44	8.4	3.45	144.0	664	12.1	37.5
2	39.4	406	10.3	2.38	10.1	4.25	105.6	429	10.7	41.3
3	32.9	364	11.0	2.14	11.2	5.24	103.2	376	11.6	47.7
4	23.8	306	12.9	1.85	11.0	5.96	101.6	311	11.2	46.3

TABLE 3

SAMPLE	MGM. S PER 100 CC. PLASMA	MGM. S PER 100 CC. URINE	CONCENTRATION RATIO OF S	MGM. P PER 100 CC. PLASMA	MGM. P PER 100 CC. URINE	CONCENTRATION RATIO OF P	URINE, CC. PER HOUR	MGM. S PER HOUR	MGM. P PER HOUR	CONCENTRATION RATIO OF P, S = 100
1	68.2	133	1.95	39.5	109	2.76	292	388	318	142
2	61.4	156	2.54	33.3	116	3.49	167	261	194	137
3	57.7	169	2.93	31.3	119	3.81	126	213	150	130
4	43.1	176	4.08	28.3	121	4.29	121	213	146	105

after the sulphate-phosphate injection was omitted in order to get a varying rate of urine flow.

Experiment 2. Body weight of dog 13.24 kilos. Intravenous injection of 10 cc. per kilo of a solution of 5 per cent anhydrous Na_2SO_4 and 5 per cent granular Na_2HPO_4 brought to pH 7.4 by addition of H_3PO_4 was started at 10:40, ended at 11:00. Bladder emptied and urine collection started at 11:00. Urine collection made at 15-minute intervals, blood sample taken at middle of each period. Results in table 3.

It is seen that the concentration ratios of S and P are more nearly the same when the plasma level of both is high than in table 2, where only the sulphate has been increased. Even in table 3, however, the concentration ratios of S and P are in no period the same, nor is their relation constant from period to period. In period 4 they agree fairly well but the fallacy of drawing conclusions from single samples of plasma and urine is well illustrated here. It will also be noted that whereas in the preceding experiments sulphate was concentrated more efficiently than phosphate, in the present experiment the reverse is true. The relative efficiency of concentration of phosphate, as compared with that of sulphate, is increased in this experiment, where the plasma phosphate level has been experimentally increased, although the absolute value of the concentration ratio of phosphate is

TABLE 4

SAMPLE	MGM. S PER 100 CC. PLASMA	MGM. S PER 100 CC. URINE	CONCENTRATION RATIO OF S	MGM. UREA N PER 100 CC. PLASMA	MGM. UREA N PER 100 CC. URINE	CONCENTRATION RATIO OF UREA N	URINE, CC. PER HOUR	MGM. S PER HOUR	MGM. UREA N PER HOUR	CONCENTRATION RATIO OF UREA N, S = 100
1	51.2	420	8.2	36.2	161	4.5	166	697	267	55
2	43.4	442	10.2	30.4	171	5.6	128	566	219	55
3	38.0	431	11.3	27.2	193	7.1	97	418	187	63
4	30.3	384	12.7	21.6	211	9.8	83	319	175	77

lower than in the preceding experiment where the plasma phosphate level was normal.

In the next experiment sulphate is compared with urea.

Experiment 3. Body weight of dog 10.40 kilos. Intravenous injection of 10 cc. per kilo of 5 per cent Na_2SO_4 and 1.5 per cent urea started at 10:10, ended at 10:30. Bladder emptied and urine collection started at 10:30. Urine collections made at 15-minute intervals, blood sample taken in middle of each period. Results in table 4.

It is seen that the sulphate is concentrated more than urea but that the concentration ratios of the two bodies do not bear a constant relation to each other from period to period.

In the last experiment sulphate is compared with sugar in the phlorhizinized dog.

Experiment 4. Body weight of dog 12.10 kilos. One gram phlorhizin per 5 kilos body weight in 1.2 per cent Na_2CO_3 solution subcutaneously at 10:30. Intravenous injection of 10 cc. per kilo of 5 per cent Na_2SO_4 solution started at 10:40, ended at 11:00. Bladder emptied and urine collection started at 11:00. Intravenous injection of 30 cc. per kilo per hour of 1.8 per cent NaCl solution started at 11:00 and continued through urine collection to promote diuresis. Urine collections made at 15-minute intervals, blood sample taken in middle of each period. Results in table 5.

Table 5 shows that sugar in the phlorhizinized dog is concentrated more efficiently than sulphate but that its concentration ratio does not stand in a constant relation to that of sulphate in the different periods. We can also see by the argument applied in the second paper (3) of this series that a true secretion of sulphate is taking place. If we consider periods 3 and 4 we see that the rates of urine flow and

TABLE 5

SAMPLE	MGM. S. PER 100 CC. PLASMA	MGM. S. PER 100 CC. URINE	CONCENTRATION RATIO OF S	PER CENT SUGAR PLASMA	PER CENT SUGAR URINE	CONCENTRATION RATIO OF SUGAR	URINE, CC. PER HOUR	MGM. S PER HOUR	GRAM SUGAR PER HOUR	CONCENTRATION RATIO OF SUGAR, S = 100
1	29.9	447	15.0	0.126	2.22	17.6	310	1385	6.87	117
2	21.8	364	16.7	0.106	1.90	17.9	280	1020	5.32	107
3	15.8	240	15.2	0.088	1.63	18.5	254	610	4.14	122
4	14.8	168	11.7	0.074	1.56	21.1	251	421	3.92	180

therefore presumably of glomerular filtration are practically the same. We should expect, then, on the filtration-reabsorption basis with sulphate a no-threshold body, $\frac{14.8}{15.8}$ or 94 per cent as much sulphate in period 4 as in 3. Ninety-four per cent of $610 = 573$ mgm. S expected in period 4. But only 421 mgm. S were excreted in period 4, or 73.5 per cent of the amount predicted. The deficit of 152 mgm. cannot be explained as due to more complete tubular absorption of S in period 4, for there is no reason to assume a more complete absorption with the same rate of glomerular filtration and at a time when the plasma sulphate level is still many more times above the normal. We must infer a tubular secretion of sulphate, more active in the third period than in the fourth. By the same line of reasoning a tubular secretion of sulphate can be shown in any of the experiments reported here.

Discussion. The results of the experiments reported here show that the elimination of sulphate in the urine is regulated by factors which are as yet quite obscure. That is, no fixed rule such as Cushny sought to apply in his "modern theory" can be assigned to the process of sulphate elimination. The same has already been shown in the two preceding papers for urea, phosphate and for sugar in the phlorhizinized dog. We cannot predict, from a knowledge of the plasma concentration of a given body and of the presumptive relative rate of glomerular filtration, as indicated by the rate of urine flow, how much of that body will be eliminated per unit time in the urine. While it is true that a high plasma content of a given constituent will effect an increased rate of elimination of that constituent, there is by no means an immutable relation between plasma concentration and rate of elimination. We realize that this last statement must not be applied arbitrarily to urinary constituents in general but that each constituent must be investigated individually. We have in these experiments only a few data bearing on the relation of plasma concentration to rate of elimination of a given body, irrespective of rate of urine flow. Addis and Drury (6) who have made extensive observations on urea excretion have concluded that, under carefully regulated conditions, the rate of elimination of urea is quite closely proportional to the plasma level. We have only one experiment in which urea excretion was followed for several consecutive periods with varying plasma urea level. The results are in table 4. An examination of these figures will show that the rate of urea elimination here is fairly closely proportional to the plasma urea level, although the correspondence is not quite so close as found by Addis and Drury. The presence in our experiment of a great excess of sulphate may have been a disturbing factor.

When, however, we turn to the other bodies studied we find no approximation to a constant relation between rate of elimination and plasma content. Thus in the case of phosphate, to turn back to tables 2 and 3 in a preceding paper (3), we see in table 2 that with a practically constant plasma phosphate level the rate of excretion had fallen from 3.95 mgm. P per hour in this first period to 0.90 mgm. P per hour in the fourth period and down to an immeasurable trace in the fifth period. In table 3 we see that with a plasma P level in the fourth period 68 per cent of that of the first, only 37 per cent as much P is excreted in the fourth as in the first. And table 3 in the present table shows a similar failure of correspondence. The same conclusion in regard to sugar in the phlorhizinized dog can be drawn from the figures of table 1 in a preceding paper (3). No consistent approximation to

constancy of this relation is shown in the sulphate figures in the present paper. For instance, in table 5 with a plasma S level in the fourth period 49.5 per cent that of first period we find only 30.4 per cent as much S excreted in the fourth period as in the first.

It may be noted, however, that the relation is much more nearly constant for sugar in the phlorhizinized dog in table 5 of the present paper. The factors which determine the extent to which this relation approximates constancy in the case of sugar are quite unknown. It may be that the approximate constancy of the relation for sugar in this case is merely a matter of chance. We hope to carry out in the future systematic observations on possible factors influencing this relation. It may be that the presence in excess in the plasma of some other body may influence this relation for a given body. Addis and Drury (7) have recently reported the disturbing effect of certain factors, as various drugs, exercise, etc., on this relation in the case of urea.

To restate briefly our conclusions, we may say that for the present we must accept the view, unsatisfactory as it may seem, that urea, phosphate, sulphate and sugar in the phlorhizinized dog come in part through the glomerular filtrate and in part through tubular secretion added to the glomerular filtrate. There is at present no evidence, for the mammalian kidney at least, as to what fraction of each body comes by each route.

SUMMARY

A modification of Fiske's technique for the determination of sulphate in urine is described, making it applicable to citrated blood or plasma.

The concentration ratio of sulphate is compared with that of urea, phosphate and of sugar in the phlorhizinized dog.

The elimination of sulphate is compared through several consecutive periods with the elimination of urea, phosphate and of sugar in the phlorhizinized dog.

Evidence is discussed leading to the conclusion that urea, phosphate, sulphate and sugar in the phlorhizinized dog are eliminated in part by glomerular filtration and in part by tubular secretion.

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THE SENSIBILITY OF THE EYE TO DIFFERENCES IN WAVE-LENGTH

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Received for publication April 27, 1923

For determining chromatic sensibility, or the sensibility of the eye to wave-length differences which give rise to differences in hue in different parts of the spectrum, two general methods have been used. The first involves the comparison of two contiguous homogeneous fields by the method of least perceptible difference or mean error; and the second consists in determining the width in wave-lengths of a portion of the spectrum which is shortened until it appears monochromatic. The second method has been particularly used by Edridge-Green (1). A review of the chief facts of hue discrimination and their relative importance is found in Parsons (2).

Early investigators found that the ability of the normal eye to detect spectral hue difference was greatest at two places in the spectrum, namely, in the yellow (570 to 595 $m\mu$) and blue-green (480 to 500 $m\mu$). Among these workers may be mentioned Lamansky (3), Dobrowolsky (4), Uhthoff (5) and Brodhun (6). Peirce (7) demonstrated another region of minimum threshold in the red at about 660 $m\mu$ and König and Dieterici (8) another one in the violet at 440 $m\mu$, later also seen by Exner (9).

The least differences in wave-length discernible as differences in hue found by these authors range between 0.8 $m\mu$ and 1 $m\mu$; in some cases the smallest threshold being in the yellow, in others in the blue-green. König and Dieterici, using the method of mean error, obtained values which are about half as large as those obtained by the method of minimal difference. It should be remarked that the matches as made by König and Dieterici are due to luminosity differences as well as to hue differences, and thus their results have no simple relation to hue discrimination.

¹ The cost of some of the apparatus was defrayed by a grant from the Loomis Fund of the Yale School of Medicine.

Steindler's (10) work is regarded as being the most complete and accurate in the subject. She found four regions of minimal thresholds at 435, 495, 585 and 636 $m\mu$ for her own right eye. Eleven other observers showed minima in the same general regions with variations in position as follows: first, 15 to 20, second 30, third 15, fourth 15 to 20 $m\mu$. Again it must be noted that Steindler's data were not taken at constant spectral luminosity. Jones (11) who has most recently investigated hue discrimination obtains results which are quite close to those of Steindler. He finds the first minimum at 445, the second at 495, the third at 586, and the fourth at 637 $m\mu$.

As to the results obtained with the method originated by Edridge-Green, it is obvious that the points in the spectrum where hue perception is best will be marked by short "monochromatic patches." These occur, according to the results reported by Edridge-Green (1), in the same general regions where others see differences in hue most easily, that is to say, in the blue-green and yellow. Houstoun (12) using this method gives figures which, on analysis, afford some evidence that there are two additional minima in the violet and orange-red, respectively.

But these so-called monochromatic patches are not really monochromatic, because if a part of such a patch is compared with a part separated from it by a difference less than the total width of the patch, a hue difference between the two parts of the patch is clearly seen. As a matter of fact, the Edridge-Green monochromatic patch appears as such because the wave-lengths included therein vary gradually over its area. If such a patch be separated into its longer and shorter wave-lengths by obscuring a middle portion, the hue differences appear plainly. This has been shown by Rayleigh (13), Watson (14) and Houstoun (12).

Edridge-Green (1, 1911) claims that other observers distinguish hue differences of a smaller number of wave-lengths than those included in a monochromatic patch because they are not careful to exclude stray white light. Watson (14), however, has shown that the addition of white light does not increase the hue perception, except possibly in the green, although Priest (15) has demonstrated that he can determine the transition of the center of gravity of two grays with an uncertainty less than 1 $m\mu$.

One of the most evident factors influencing hue discrimination is that of the relative luminosity of the two wave-lengths. It was mentioned above that the matches as made by König and Dieterici are in

part due to luminosity differences. Peirce (7) pointed out in this connection (an observation which had been made before) that of two patches of monochromatic light of the same wave-length composition the brighter was the yellower, and that the dimmer light could be made to match the brighter in hue by either increasing or decreasing the wave-length. He showed that the "center" of the spectrum (582 $m\mu$) when sufficiently brilliant is whiter than a dimmer patch of the same wave-length. Abney (16) shows that if white light be added to spectral light the change in hue is of the same nature. It is therefore obvious that, if the ordinary spectrum conditions are used, intensity as well as hue vary from point to point. The luminosity must be equated at each wave-length before the hue of two wave-lengths is discriminated. This was not done, so far as we have been able to determine, by the early observers, being neglected by Peirce himself. Jones (11) maintained an intensity balance between his two fields. Steindler (10) and König and Dieterici (8) regarded this factor of luminosity as being of importance only at the ends of the spectrum. Steindler asserted, however, that the two minima at the ends of the spectrum were not due to luminosity. This failure to equate the luminosity led her into serious error in estimating the hue discrimination of the protanope.

The determination of the sensibility to hue differences is only one of the many characteristics of vision which require further statistical investigation. The first incentive to study this characteristic was given us several years ago by reading the reports by Nutting (17). The importance as well as the interest in such work is cogently indicated by Priest (15). Aside from determining the "normal" discrimination sensibility for hue the study of the influence upon the normal of the following variables is planned and in part under present investigation: 1. *Intensity*, that is, as to whether the positions of the minima shift with varying intensity. It has been previously shown that hue sensibility is nearly independent of intensity over a wide range, being only somewhat higher for brightness of medium value than for those of extremely high and low values. 2. *The size of the field*. 3. *The order in which pairs of wave-lengths are presented to the observer*. Some influence has been observed. 4. *The effect of selective and of general fatigue, both slight and severe*. The effect of selective fatigue manifested itself during the course of observations here to be described and will be referred to later. It is connected with the question of the order in which the tests are presented. The influence of severe fatigue has been studied and will be reported in another paper.

It is clear from the results hitherto obtained that observations on a number of individuals are required. The curve expressing chromatic sensibility varies considerably with the individual and we have evidence that it varies *within* the individual, probably owing to such absolutely uncontrollable factors as slight fatigue, adaptation, etc., which may be regarded as accidental variations. The relation of the hue discrimination minima to the course and intersections of the fundamental sensation curves is of particular interest in this connection. The ability of those with defective vision to discriminate hue throughout the spectrum should also be reinvestigated. We have been fortunate in being able to make a careful set of tests on a dichromat (a protanope) under "normal" conditions of retinal adaptation, etc., as well as after fatigue.

The present paper is one of a series of contributions to a statistical determination and correlation of the characteristics of vision.

Apparatus. Two forms of apparatus were used in the present study. The first consisted of two Hilger wave-length spectrometers, constant deviation type (see fig. 1). The telescopes T_1 , T_2 , from which the inner tubes carrying the oculars were removed, were equipped with slits, S_1 , S_2 set at the focal point of the D lines, the slits being just wide enough to allow both of them to pass. The collimator slits were set and kept throughout at such a width that the two D lines just fused into a single band.

The spectrometers were so placed that the telescopes were at right angles and their slits as close to one another as possible. These were viewed through an ocular, OC , after having been brought into vertical juxtaposition, by means of a small cube, LBC , of the Lummer-Brodhun type. The field visual angle was $12'$ by 3° .

Two 100 watt, Mazda C lamps, L_1 , L_2 , were used as sources of light. They were mounted in a horizontal position, so that the filament of each was vertical in front of the collimator slits. The intensity was balanced by varying the distances. This was done by the observer by means of pulleys and cords, the boxes containing the lamps running on tracks. With this apparatus data on seven subjects were obtained. Owing to certain disadvantages it was eventually given up in favor of the one shown in figure 2. The telescope tube of each spectrometer was shortened by unscrewing the section beyond the binding support. A Lummer-Brodhun cube, LBC , was set with its center equidistant from the two telescope lenses so that the light from each source was brought to a focus at the pin hole eye piece, P . With the drums set

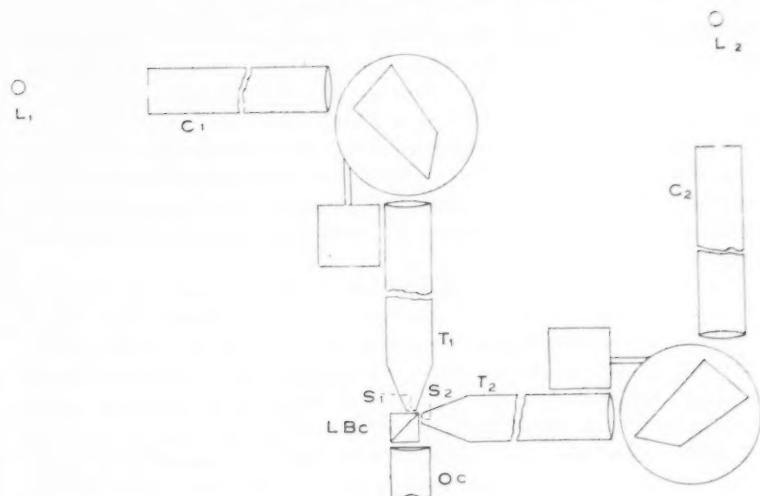


Fig. 1

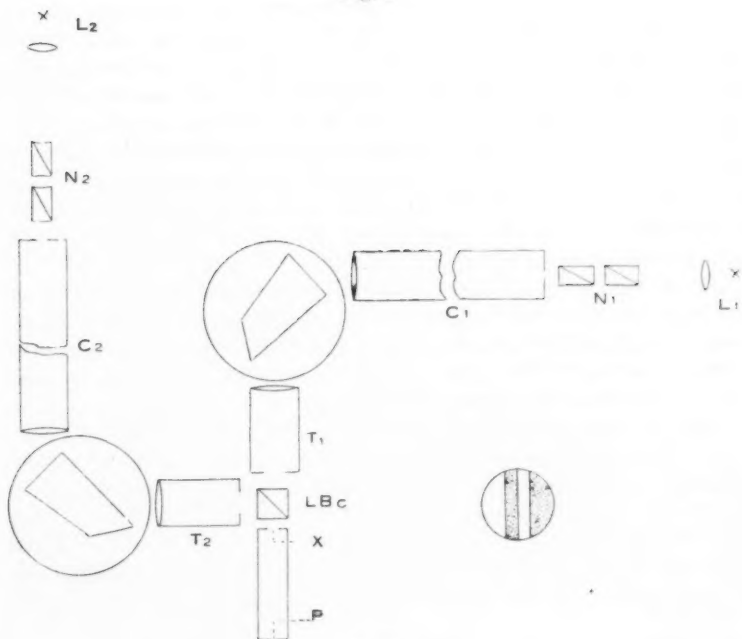


Fig. 2

at 5893 the two sets of sodium lines seen through a Ramsden ocular were superimposed the one upon the other and centered in the pin hole aperture, 0.38 mm. in diameter. The field had the form shown in the insert in figure 2. Its size (3°) was determined by a diaphragm placed at X. The dotted area emanated from one of the spectrometers, the clear from the other. When the instruments were set so as to deliver the same wave-lengths and the luminosity differences equalized, the pattern disappeared, the field becoming a homogeneous circle.

Two 500 watt concentrated filament Mazda C lamps, L_1 , L_2 were used as the sources of light. The intensity was controlled by Nicol prisms, N_1 , N_2 manipulated by connecting rods in the hands of the subject. Stray light in the optical system and that reflected from the inside of the telescope tubes was cut down to a minimum by introducing blackened diaphragms, gradually decreasing in size of opening, into the telescope. The observer was carefully screened from all direct light other than that passing through the spectrometers and always remained, before observations were begun, for a constant length of time in the dim light of the experimental dark room.

Methods. The data were obtained by determining the least change in wave-length which gave rise to a sensation of hue difference after the luminosity difference had been equated. The psycho-physical method used to obtain the data was in all essentials one of answers. An assistant presented to the subject a pair of lights slightly different in wave-length. The subject then equated the luminosities and gave a decision as to whether the two fields were of the same hue or not. According to the answer a greater or less difference in wave-length was presented until the least perceptible difference was found. In order to avoid the effect of subconscious expectation the order of presentation was varied. Furthermore the longer of the two wave-lengths might appear on either part of the field and the subject was required to say which. The tests were also presented in as irregular an order as was compatible with the avoidance of fatigue.

The spectrum was scaled continuously, discontinuously and at random. The first involved the measurement of each least perceptible difference in hue in the spectrum between 400 and 700 $m\mu$ proceeding in one set of tests from the red end to the blue, and in another set in the reverse order. The second involved the determination of the least perceptible difference in hue at intervals of 10 $m\mu$ and at intervals of 5 $m\mu$, again proceeding in individual series in both directions. The

luminosities of successive pairs of lights were always so adjusted that they were of equal brilliance throughout the spectrum. As a final check on the results obtained by the continuous and the discontinuous scaling, the method of random selection of two pairs of lights was done. By the continuous and discontinuous methods a luminosity curve was established. The values of the luminosity at any wave-length could thus be read off from a table and the brightness of the fields set at that point. A method of mean error was also used, but not carried very far. It consisted in setting one of the spectrometers at a chosen wave-length, and then requesting the subject to match this color in the other spectrometer by moving the wave-length drum. The results obtained in this way were in great measure due to luminosity differences and are not true indices of the discrimination of hue differences.

In all of our experiments the field was at a constant brightness throughout the spectrum. The value for this with the first apparatus was 1.5 millilamberts, for the second a retinal illumination of 4 photons (18).

Results. The determinations with the first apparatus were made on seven subjects; with the second apparatus on four, two of whom in each case were the authors.² The results obtained by the continuous method will be given first. The spectrum was stepped off according to the difference threshold as the unit, in one set of cases proceeding from the red to the blue and in another from the blue to the red. The average results for ourselves are shown in figure 3 and table 1. They are in general, similar to those of Steindler (see the average curve given by Nutting (19) and of Jones (11)). The number of hues seen by L. is 161 and by H. 207. The complete tabulated results giving the difference thresholds at each step have been omitted in the interests of economy, and the chief results will be shown by curves which are moreover better adapted to give a clear idea of the results than are columns of figures. Abbreviated tables are given showing the position of the minima (that is, the places where the difference threshold or delta is smallest or the sensibility is at a maximum) and of the maxima (where the delta is greatest and the sensibility at a minimum) together with the value of the delta in $m\mu$. The values were obtained by taking in each case the position and value in $m\mu$ of the minima and averaging

² We take pleasure in here recording our thanks to the following who served as subjects: Miss A. E. Adams, Miss E. E. Allis, Miss E. F. Botsford, Miss Florence Dowden, Miss Edith Lowman, Mr. W. M. Copenhaver, Mr. C. E. Packard, Mr. A. Sperandeo.

them, similarly for the maxima. A curve based on such averages for 5 subjects is given in figure 4 and table 1.

One possibly objectionable feature of scaling the spectrum continuously is that the entire series of observations necessary to complete the set consumes several sittings. Our experience has been that after an hour, on the average, hue perception begins to weaken so that false results are obtained if the observations are continued longer.

TABLE I

Location and value of the positions of maximum and minimum hue sensibilities

SUBJECT	FIRST MINIMUM	FIRST MAXIMUM	SECOND MINIMUM	SECOND MAXIMUM	THIRD MINIMUM	THIRD MAXIMUM	FOURTH MINIMUM
Continuous method							
H.....	440 0.62	460 1.37	490 0.25	532 2.25	585 0.50	605 1.37	620 0.75
L.....	440 1.87	455 2.87	482 0.75	535 2.25	578 0.37	618 2.25	630 1.75
Average of 5 subjects.....	448 0.75	468 2.12	482 0.50	529 2.75	579 0.50	621 3.00	631 2.30
Discontinuous method at 10 m μ , first apparatus							
C.....	442 0.66	457 1.12	476 0.66	517 1.50	570 0.75	608 1.87	631 1.30
H.....	440 1.00	458 1.56	484 0.45	516 1.40	565 0.35	600 1.66	630 0.66
L.....	437 1.12	455 1.75	482 1.12	520 2.25	586 1.12	612 2.12	630 1.25
P.....	443 0.83	452 1.12	475 0.67	530 2.00	570 1.00	600 2.75	628 1.16
Average of 4 subjects.....	441 0.90	455 1.39	479 0.72	521 1.79	573 0.81	605 2.10	630 1.27
Second apparatus, average of 16 series							
H.....	440 1.06	470 2.09	496 0.99	536 2.47	587 0.71	611 1.82	623 1.30
L.....	433 1.19	455 2.70	486 1.41	526 3.34	580 0.97	609 2.39	630 1.64
Discontinuous method at 5 m μ , first apparatus							
Average of 6 subjects.....	448 0.96	458 1.60	480 0.75	525 2.00	581 0.45	615 1.90	628 1.25

The results with the first apparatus by the discontinuous method, in which the delta at every 10 m μ was found are shown for four subjects, figure 5 and table 1. Note should be made of the fact that these curves are flatter than those obtained by the continuous method. In these observations, as in the continuous method, the spectrum was scaled in both directions. A complete set of observations could be made in one sitting lasting about an hour.

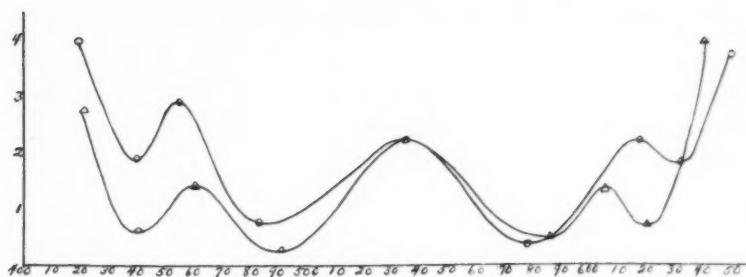


Fig. 3. Hue discrimination curves for H (triangles) and for L (circles) by the continuous method, first apparatus. In this and in all later figures, abscissae are wave lengths in $m\mu$, ordinates are the least perceptible difference (difference threshold) in $m\mu$.

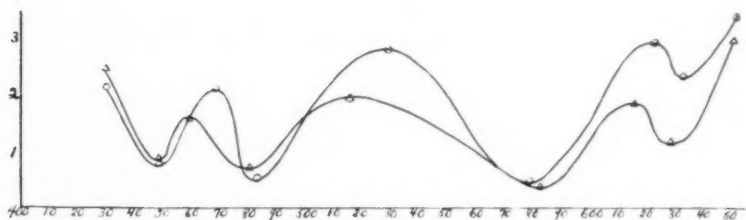


Fig. 4. The average discrimination curves of five subjects, continuous method (circles); and for six subjects, discontinuous method at every 5 $m\mu$ (triangles).

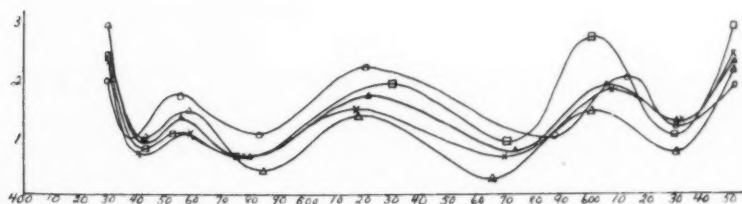


Fig. 5. Discrimination curves for each of four subjects, and the average (in solid triangles). Discontinuous method at 10 $m\mu$.

The results with the second apparatus are particularly valuable since they represent the average of 16 determinations for each of two subjects. Some of the individual curves did not come out as smoothly as others and the position as well as the values of the maxima and minima are not invariably the same for the same subject. These variations will be referred to again later.

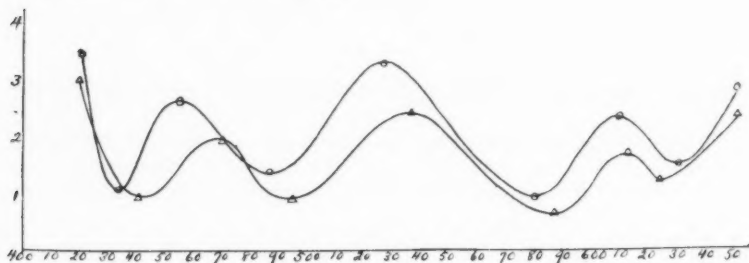


Fig. 6. Discrimination curves, each of which is the average of 16 complete series, of H (triangles) and L (circles). Discontinuous method at 10 mμ.

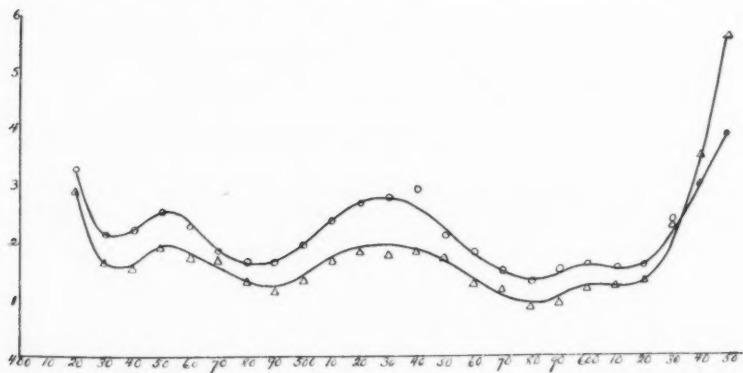


Fig. 7. Discrimination curve based on the simple averages of the values of the difference threshold at every 10 mμ, 16 tests on each of the authors. Symbols as in figures 3 and 6.

These curves are shown in figure 6 and table 1. For comparison, curves plotted from a simple average of the deltas at every 10 mμ are given in figure 7. It will be noted that, while these latter show the general characteristics of hue discrimination curves, they are flatter.

This is due to the slight lateral shift in the positions of the minima and maxima which results in lowering the average. The positions of the first and fourth minima seem especially subject to this shift. Curves based on the averages of values at every $10\text{ m}\mu$ which have been obtained by the continuous method are similar to those shown in figure 7.

The spectrum was also scaled to find the delta at every $5\text{ m}\mu$ on six subjects using the first apparatus. The average curve is shown in figure 4.

In connection with the question of the order in which the tests are presented as well as the influences of fatigue it was deemed inadvisable to determine the difference limen at every $10\text{ m}\mu$ but to select pairs of wave-lengths in the spectrum at random. There was opportunity

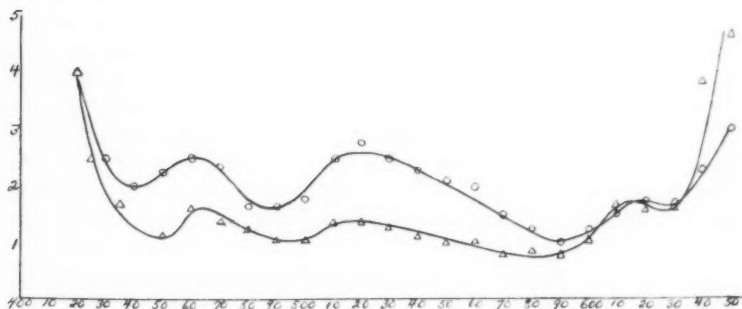


Fig. 8. Discrimination curves based on the simple averages of the values of the difference thresholds at every $10\text{ m}\mu$. Determinations made at every $10\text{ m}\mu$ but not in sequence. Symbols as in earlier figures.

to carry out such determinations on two subjects only, namely, the authors, but this was done at least five times for every $10\text{ m}\mu$ and for many such points as often as seven, eight or nine times.

The idea in making such hue discrimination tests at points selected in the spectrum at random, that is, not in regular procession through the spectrum, was that the influence, if any, of the previous test might be eliminated. It was considered possible that some such factors as contrast and adaptation (fatigue induced by the preceding pair of lights, etc.), might affect the discrimination threshold.

The curves are reproduced in figure 8. Being based on an average of all the values at every $10\text{ m}\mu$ the resultant curves are necessarily flatter than those obtained by averaging only the positions and values

of minima and maxima. Similar conditions as mentioned above were obtained in averaging the values found at every 10 $m\mu$ by the discontinuous method.

The point must be again emphasized that the actual positions of the maxima and minima shift somewhat in successive determinations for the same individual; also that the actual values at the same wave-lengths may vary more or less. When such results are averaged, there is consequently produced an average curve which does not express the results which would actually be obtained in any one test.

It is clear that the statistical average taken from many observations must be used. Great accuracy is particularly unobtainable in all measurements at the limit where a phenomenon is visible before it vanishes. All kinds of uncontrollable changes in the condition of our nerve apparatus and psychical activity come into play, and become apparent finally in the variation of the results of the measurement. This variability was also met with in the assignment of color names to wave-lengths. There can be no doubt that the relative magnitude of the three independent variables or elementary processes must vary from time to time according to the state of retinal adaptation at the time, assuming also, what is very probable, that nearly all spectral lights stimulate to some extent all three of the fundamental color processes.

Steindler has pointed out that the hue discrimination curve follows quite closely, at least as far as the position of the minima are concerned, the resultant of the slopes of the three sensation curves as calculated by Exner. Hue is determined by the relation of the ordinates of the fundamental sensation curves in the particular part of the spectrum. A rapid change in these relations in neighboring parts of the spectrum results in rapid changes in hue and a place where such a change takes place must be a place of high sensitivity. But if the ordinates of the fundamental sensations in two neighboring parts increase in an approximately proportionate manner, then the hue of this region will change but slowly and such a place is one of low sensitivity.

Beginning at the red end of the spectrum hue discrimination is seen to be determined by the shape of the green sensation curve. In proportion to the increase in the ratio of green to red sensations, the color becomes more yellow (orange). (See table 3.) The proportion of green begins to increase rapidly at the fourth minimum (620 to 630 $m\mu$). Below this, the red and green sensation curves run nearly parallel, a region of lower sensibility, until the red curve reaches its

crest and then going down, crosses the green sensation curve which is still rising. At this point in the spectrum the contrast is between a reddish yellow (golden yellow) and a greenish yellow. This is the location of the third minimum (565 to 587 $m\mu$). The entrance of the blue curve adds white to the shorter wave-length as it is seen in contrast to one slightly longer. This effect is reversed below the intersection of the blue and red curves. The whiteness is less with decrease in wave-length and declines with the red curve (16, p. 243, fig. 84). The second minimum (480 to 496 $m\mu$) occurs at or near (20, pp. 60 and 214) the intersection of the blue and green curves, and therefore is of the nature of a contrast between greens containing more or less blue (blue blue-green or green blue-green). The first minimum (433 to 448 $m\mu$) is seen as a contrast between blue and violet (blue blue-violet or violet blue-violet) and must be due to an increase in the red sensation as well as to a decrease in the green sensation as we pass below 460 $m\mu$ (16, p. 240).

One of the most interesting things in connection with the variation in results is the presence, when the spectrum is scaled from blue to red, of a minimum delta near 520 $m\mu$. In some cases this was as small a threshold as that occurring at the second or third minimum. It did not appear when the spectrum was scaled from red to blue.

In figure 9 curves (circles and triangles) based on the simple averages of deltas obtained in going from the blue to the red end of the spectrum are given to illustrate this point. The curve in crosses is of a single series of determinations. That the average curves (of 8 series for each observer) show this minimum at 520 $m\mu$ indicates that the phenomenon is of quite regular occurrence. Furthermore, a minimum at this point is to be seen in some of the blue to red curves of all of our observers.

This minimum at or near 520 $m\mu$ is explicable in terms of the relative slopes of the three sensation curves. The red and blue sensation curves cross near wave-length 520 $m\mu$ (16, p. 369). If, due to selective fatigue, the intensity of the green sensation is low, the contrast between the red and blue sensations, which should be very great here, becomes manifest. This point was proved by fatiguing the eye with a bright green (517 $m\mu$ at 52 millilamberts) and then testing hue discrimination. The usual minima (with the exception of the first) were obliterated and the curve sloped to a minimum at 520 $m\mu$ (see the following article). A plausible reason for the appearance of the minimum at 520 $m\mu$ when the spectrum is scaled in one direction,

namely, from blue to red, and not when scaled in the other, is found in the excessively low stimulus value (luminosity) of the blue sensation (21). Nearly all the brightness of the blue green as short as $490\text{ m}\mu$ is due to the green sensation. Whereas on the *long* side of $520\text{ m}\mu$ the green plays a relatively small part in the stimulus.

To recapitulate this argument briefly, at the regions of high and low hue sensibility there are rapid and slow changes respectively in the relations of the fundamental sensations. Difference in hue is determined by the differences between the relations of the fundamental processes set up in the eye. This further offers an answer to the ques-

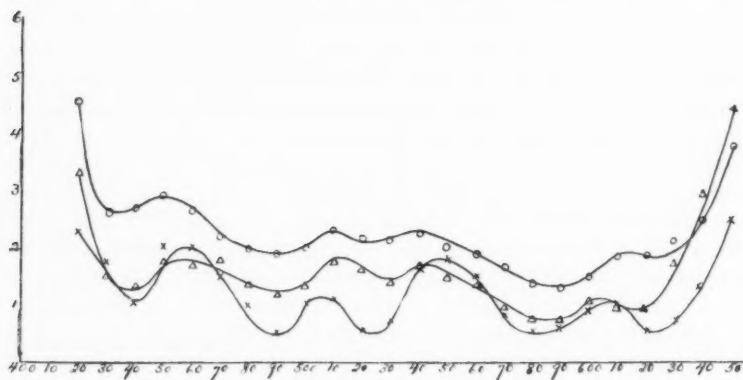


Fig. 9. Discrimination curves (circles and triangles) based on the difference limen at every $10\text{ m}\mu$. Determinations made in blue-red sequence. Note the lowering of the curve near $520\text{ m}\mu$. The curve designated by crosses is a single typical case.

tion as to why the wave-lengths of high and low sensibility are different for different observers. The wave-lengths of the average points of individual fundamental curves vary, that is, the red, green and blue values of a homogeneous color sensation at the same part of the spectrum are not the same for all observers. Furthermore, as mentioned above, to the fluctuations of the fundamental sensation curves occasioned by the variation in the magnitude of the three processes are to be attributed the individual differences in sensibility shown by the same eye.

The naming of spectral colors. To throw further light upon the nature and cause of this variability in hue discrimination and its rela-

tion to the three fundamental sensations the names given to the various parts of the spectrum, as they were presented one after the other in orderly sequence through the spectrum in both directions, were studied (table 2). The names of two contrast colors as they appeared when the wave-lengths were set at the point of least perceptible difference were also noted (table 3). The results for the naming of one color are given in table 2 for a bright, medium, and dim spectrum. The wave-lengths placed in line with the color names are in all cases the longest wave-length to which the given name was applied. Thus, as shown in column 2, the observers, on the average, gave the name red to the long wave-lengths as short as $637.5\text{ m}\mu$; orange-red to those from 637.5 to $614.4\text{ m}\mu$.

As might be expected, there is considerable variation among the subjects and undoubtedly a similar variation in the same eye from day to day, due to uncontrollable physiological factors (table 2, columns 3 and 5). One of these physiological factors, the results of which seem fairly constant, is successive contrast, in terms of the sequence in which the colors are viewed. As a consequence there is a shifting of the color name series in relation to the wave-length series in a direction opposite to that in which the colors of the spectrum are presented. For example, when going from red to blue, wave-lengths 595 to $575\text{ m}\mu$ are called yellow; whereas when going in the opposite (blue to red) direction, these wave-lengths are seen as shades of orange and red, and yellow has shifted down to 557 to $531\text{ m}\mu$. This is a rather extreme case. The table, however, shows that the shift is general. It is decidedly less in the case of a weak spectrum than in a strong one. It is less with the newer apparatus than with the first, but it is qualitatively the same. This investigation of color naming is to be repeated with regard to the size of field, brightness, and the intervals between and during exposure of the retina to the light.

The position of the hue discrimination minima do not shift against the direction in which the wave-lengths are presented. In the early stages of this investigation it was thought that there was a marked shift in the same direction as the presentation. This was found later to be due to the appearance of the minimum at $520\text{ m}\mu$ (discussed above) when the spectrum was scaled from blue to red, and a simultaneous disappearance of the first minimum. The idea of a shift in the position of the minimum was shown to be fallacious when we obtained "blue to red" curves showing five minima, that is the usual four and, in addition, the one at, or near, $520\text{ m}\mu$.

TABLE 2

Names given to the colors of the spectrum when seen in sequence from blue to red and from red to blue. The wave-lengths are the longest to which the name opposite was given

NAMES OF COLORS	RED TO BLUE		BLUE TO RED	
	Average for red end of color	Average variation	Average for red end of color	Average variation
20 millilamberts at 590 m μ ; 7 observers				
Red end	772.5	± 14.2	750.0	± 6.0
O-R	637.5	12.5	601.9	15.2
O	614.4	15.6	585.6	11.7
O-Y	602.8	13.9	571.2	12.5
Y	591.9	9.4	557.5	13.8
Y-G	575.0	10.0	531.3	18.8
G	532.5	14.4	507.1	12.4
B-G	505.6	12.1	488.6	5.9
B	481.9	11.9	465.6	13.8
B-V	461.9	8.4	447.9	4.4
V	443.8	10.0	431.3	6.8
End	405.1	4.9	410.6	5.7
5 millilamberts at 590 m μ ; 4 observers				
Red end	705.0	± 10.0	726.3	± 11.3
O-R	626.3	8.7	596.3	1.8
O	598.7	3.8	586.3	3.8
O-Y	591.2	4.1	565.0	10.0
Y	583.7	1.9	553.8	6.7
Y-G	576.3	3.8	536.3	16.3
G	551.3	4.1	518.8	18.8
B-G	525.0	15.0	501.3	18.8
B	493.8	13.8	471.3	14.4
B-V	477.5	2.5	455.0	35.0
V	470.0	0	438.3	22.2
End	425.0	23.8	421.6	15.5
1.25 millilamberts at 590 m μ ; 2 observers				
Red end	700.0	± 0	705.0	± 15.0
O-R	630.0	10.0	600.0	0
O	597.5	7.5	595.0	0
O-Y	592.5	7.5	585.0	10.0
Y	582.5	2.5	555.0	0
Y-G	575.0	5.0	550.0	0
G	547.5	7.5	535.0	35.0
B-G	535.0	5.0	517.0	17.0
B	495.0	10.0	477.5	2.5
B-V	480.0	0	470.0	0
V	470.0	0	445.0	0
End	432.5	32.5	430.0	0

This apparent independence of color names and hue discrimination might be regarded, in the light of the Theory of Zones (22), as indicating an independence of cerebral and retinal function. From a physiological point of view, this attitude may be criticised as neglecting the factor of simultaneous contrast between colors presented for discrimination. Our records (see table 3) are not complete, but, as stated above, the first minimum is a result of contrast between blue and violet; the second between blue-green and blue, or between blue-green and green; the third between yellow-green and yellow; and the fourth between orange and red. There is no distinct difference between the colors seen at the several minima when the spectrum is viewed in the

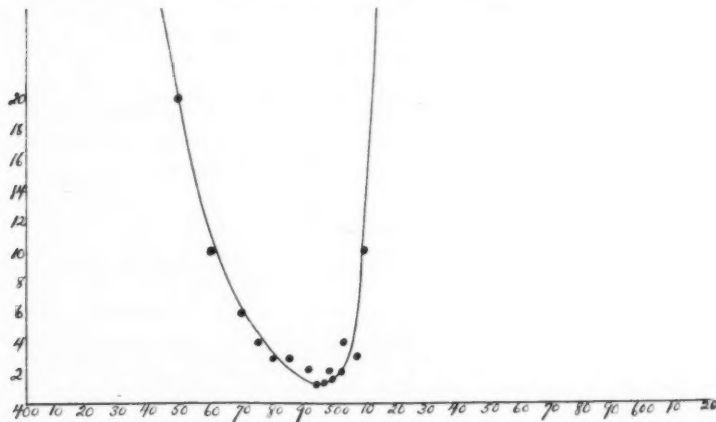


Fig. 10. Hue discrimination curve of a protanope.

two different directions. Simultaneous contrast has wiped out the shift due to successive contrast.

Thus table 3 shows that, when two wave-lengths in the neighborhood of 620 $m\mu$ are contrasted, one appears orange, the other yellow; whereas if seen singly (table 2) when the spectrum is presented in the blue to red sequence, 620 $m\mu$ appears red, and when in the red to blue sequence, orange-red. In spite of the fact that the contrast colors were seen in a red to blue sequence, the long member of the pair shows a shift toward the short end of the spectrum due to simultaneous contrast. This shift is usually greater than the shift due to successive contrast illustrated in table 2. The short member shows a corresponding shift in the opposite direction.

TABLE 3
Contrast colors as named when the fields were set at the least perceptible difference. Spectrum scaled from red to blue

WAVE- LENGTHS	SUBJECT L							
	Series 1	Series 2	Series 3	Series 4	Series 1	Series 2	Series 3	Series 4
mμ								
650	R:R-O	R:O	R:O	O:Y	R:O	R:O	R:O	R:O
640	O:O-Y	R:O	R:O	O:Y	R:O	R:O	R:O	R:O
630	O:O-Y	R:O	R:O	O:Y	R:O	O-R:O	O:Y	O:Y
620	O:Y	O:Y	R:O	O:Y	O:Y	O:Y	O:Y	O:Y
610	Y-O:Y	O:Y	O:Y	Y:G	O:Y	O:Y	Y:G	Y:Y
600	Y:Y-G	O:G	O:Y	Y:G	O:Y	O:Y	Y:G	Y:Y-G
590	Y:G	O:G	O:G-Y	Y:G	O:Y	O:Y	Y:G	Y:Y-G
580	Y:G	O:G	Y-O:G	G:Y	O:Y	Y:G	Y:G	Y:Y-G
570	Y:G	Y:G	Y:G	Y:G	Y:G	Y:G	Y:G	Y:Y-G
560	Y-G:G	Y:G	Y-G:B-G	Y:G	B:B-G	Y:G	Y:G	Y:Y-G
550	Y-G:G	Y-G:B-G	B-G:Y-G	Y:B-G	B-G:B	G:B-G	Y:G	Y:Y-G
540	Y-G:B-G	Y-G:B-G	G:B-G	Y-G:B-G	B-G:B	G:B-G	Y:G	Y:Y-G
530	Y-G:B-G	Y-G:B-G	G:B-G	Y-G:B-G	B-G:B	G:B-G	G:B-G	Y:G
520	Y-G:B-G	Y-G:B-G	G:B-G	Y-G:B-G	B-G:B	B-G:B	G:B-G	B-G:B
510	Y-G:B-G	G:B-G	B-G:B	B-G:B	B-G:B	B-G:B	G:B-G	B-G:B
500	B-G:B	B-G:B	B-G:B	B-G:B	B-G:B	B-G:B	B-G:B	B-G:B
490	B:V	B-G:B	B-G:B	B:B-V	B-G:B	B-G:B	B-G:B	B:V
480	B:V	B:B-V	B:V	B:V	B:V	B:V	B:V	B:V
470	B:V	B:B-V	B:V	B:V	B:V	B:V	B:V	B:V
460	B-V:V	B-V:V	B:V	B:V	B:V	B:V	B:V	B:V
450	B-V:V	B-V:V	B:V	B:V	B:V	B:V	B:V	B:V
440	B-V:V	B-V:V	B:V	B:V	B:V	B:V	B:V	B:V
430	B-V:V	B-V:V	B-V:V	B:V	B:V	B:V	B:V	B:V
420	B-V:V	B-V:V	B-V:V	B-V:V	B:V	B:V	B:V	B:V

Furthermore on theoretical grounds it may as justly be held that the apparent lack of interdependence of the color name series and hue discrimination does not lead to the conclusion that one or the other may be relatively independent of retinal processes. It may be that the two functions are dependent upon two aspects (stimulus value and change in relative stimulus value) of the sensation curves which are relatively independent of each other as regards relative fatigue or color adaptation. The name applied to a wave-length depends upon the *relative height* (stimulus value) of the three sensation curves at this point. This, as will be shown in another paper, varies with selective fatigue. On the other hand, the minima of the hue discrimination curve correspond to the regions of greatest change in the relative ordinates of the sensation curves. A change in the shape of a sensation curve, aside from its height, involving a shift of its ends or peak or the *relative declivity* of the limbs, could only be brought about by a change in the receptor process. The result is that we find the minima at, or near, their normal places or absent altogether. This point will be brought out more fully in another paper in connection with monochromatic fatigue.

A further interesting, though theoretical, point is seen on inspection of table 3. In the region of the green and yellow, and to some extent as far down as the blue-green, it is seen that the names given to the contrasting colors are rather far apart in the hue scale. Orange is seen in contrast with green in spite of the fact that the wave-lengths are set at the point of least perceptible difference. At other points colors separated by a similar "psychological unit" lie side by side on the hue scale. This has bearing upon the much argued question as to whether or not least perceptible differences are real psychological units. Certainly they vary on the subjective as well as on the objective side. The question, however, is metaphysical.

The hue discrimination of a dichromat is included in the present study. He is a protanope, his spectrum being decidedly shortened at the red end and the wave lengths of maximal luminosity (gas filled tungsten prismatic spectrum) are in the neighborhood of 545 to 535 $m\mu$, 650 $m\mu$ is much dimmer to him in comparison with 550 $m\mu$ than it is to the normal. This evidence points to the assumption that he lacks the normal red fundamental process.

In naming colors this dichromat uses the term blue correctly. He calls violet purple, greenish-purple, or green. He applies the names green, yellow, orange, red, grayish-green, grayish-red, etc., indis-

criminally to the parts of the spectrum of longer wave-length than 500 $m\mu$. The neutral gray region extends from 470 to 480 $m\mu$ when the spectrum is viewed from blue to red and from 500 to 510 $m\mu$ when the direction is reversed.

There is no shift depending upon sequence in the position of the minimal hue discrimination delta. His curve is shown in figure 10. There is little or no ability to discriminate color except between 460 and 500 $m\mu$. This is what might be expected from a survey of König's sensation curves for protanopes (20, p. 69), for in this region only do the curves change in their relative heights to any considerable degree.

Steindler, however, figures a maximum for the protanope at 600 $m\mu$. This agrees with what she was led to expect from the resultant of the hue discrimination curves. Moreover, in the case of the deuteranope she was led, by a similar calculation, to expect a maximum at 635 $m\mu$. This could not be substantiated because of lack of luminosity in her apparatus. We believe that there are errors in her theoretical calculations as well as in her experimental method. The "long" curve of the deuteranope is steepest at about 635 $m\mu$, whereas that of the protanope is steepest near 600 $m\mu$. This must be her basis for placing the dichromatic minima at these points, because the "blue" curve, which is identical for protanopes and deuteranopes, ends at 585 $m\mu$, and rises very slowly from there to 525 $m\mu$. There can therefore be no true color differences above 590 $m\mu$.

The steepness of the sensation curves in the above mentioned regions means of course rapid changes in luminosity and not change in hue. Steindler did not, as far as we can judge from her paper, regulate luminosity except as a check at the ends of the spectrum and on her own eye. It is well known that color-blind persons mistake differences in brightness for differences in color. Our subject when given two wave-lengths in the neighborhood of 600 $m\mu$ would call one red and the other green. This is obviously due to their inherent spectral luminosity differences, because when brought to equal brightness, they appeared identical as to hue.

It seems perfectly natural that the hue discrimination minimum for deuteranopes and protanopes should be in the general region of 500 $m\mu$, and slightly lower for the protanope, though this might be obscured as a result of variations in macular pigmentation. Color adaptation does not cause a shift of the minimum, but when the comparison lights are dim there is an increase in the hue discrimination threshold and a shifting of the minimum into the violet.

SUMMARY

1. A careful series of hue discrimination tests has been made in such a way that the subject has been covered statistically and with variations in sequence so as to rule out fatigue, successive contrast, etc.

2. Hue discrimination is at its best in the yellow and blue-green. In the orange and violet there are two secondary minima where the difference threshold is slightly higher. Tables and curves are given to show the values for different observers and with different procedures.

3. When the spectrum is scaled from blue to red, a fifth region of minimal difference threshold is found near $520\text{ m}\mu$ (in the middle of the green). This also appears after fatigue by strong homogeneous green light.

4. The four regions of minimal threshold, as earlier pointed out by others, correspond to the regions of greatest change in the relationship between the ordinates of the three curves representing the response of the trireceptor mechanism as experimentally determined by König, Exner, Abney and others. Our results are further evidence that the activity of the receptor apparatus is the resultant function of three independent variables.

5. The fifth region of minimal difference threshold, discovered by us, corresponds to the region in the spectrum of greatest change in the relationship of the ordinates of the red and blue (or violet) curves. It is seen only after the intensity of the green sensation has been lowered by selective fatigue.

6. There is only one minimum (at about $493\text{ m}\mu$) in the protanopic hue discrimination curve, and not two as reported by Steindler. This finding is shown to be in agreement with theoretical expectations.

7. There is a decided shift in the names applied to spectral wave-lengths due to successive induction, and thus depending upon in which direction the spectrum is scaled. No such shift is observable in the regions of minimal threshold.

8. When contrast colors are set at "least perceptible difference" they sometimes and at some wave-lengths, appear much more different from each other than they do at other times or at other wave-lengths. This subjective fact bears no relation to the physical value of the threshold in $\text{m}\mu$, but it casts doubt upon the value of the "least perceptible difference" as a psycho-physical unit.

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THE SENSIBILITY OF THE FATIGUED EYE TO DIFFERENCES IN WAVE-LENGTH IN RELATION TO COLOR BLINDNESS

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Received for publication April 27, 1923

The effect of retinal fatigue, selective and general, upon the sensibility of the eye is familiar to everyone in connection with successive contrast. The literature is voluminous, but attention will be called here to merely a few papers having special bearing upon the ability to discriminate hue (1).

Burch (2) using a very strong fatiguing light has shown that the eye can be put in a state that is similar to complete red, green, blue or violet blindness, depending upon whether the fatiguing light is red, green, blue or violet. From this he concludes that color vision is served by four fundamental sensations, red, green, blue and violet. Burch's evidence for a fourth or violet sensation has been questioned by Abney (3) and Rayleigh (4). It does not seem to hold for the average eye.

According to Burch, certain parts of the spectrum, namely, yellow and blue-green, exert a fatiguing effect upon the eye which is different from that of red, green or blue. These portions of the spectrum are in the regions where two sensations, according to the curves representing the three elementary processes, as determined by König, Abney and others, are evoked more or less equally. In such cases the wave-length in the test spectrum corresponding to the fatiguing light is not changed in hue, as it is when other parts of the spectrum are used. When the fatiguing light is moderately strong yellow, the red, yellow and green of the test spectrum are all reduced in intensity and saturation but suffer no further change. If, however, the yellow fatiguing light is very strong, the red and green are both eliminated from the test spectrum and with them of course the yellow. The same is true of blue-green in relation to the blue and green parts of the test spectrum.

¹ The cost of some of the apparatus was defrayed by a grant from the Loomis Fund of the Yale School of Medicine.

On the other hand, if the fatiguing light is weak red, blue or green, these indifferent points (yellow and blue-green) change their hue, the yellow shifting toward the middle of the spectrum after fatigue with red and toward the red after fatigue with green. If the fatiguing light is strong red, the yellow and red disappear entirely from the test spectrum (except for "dazzle tints"). The blue-green behaves in an analogous manner with relation to fatigue with blue and green light, shifting, when moderately fatigued with blue to the middle, with green to the violet end, and disappearing entirely when the fatiguing light is intense.

Abney (3) has matched homogeneous patches of light of which one is seen with an eye fatigued to red, green or blue light, the other by the unfatigued eye and thus has worked out the effect of fatigue in terms of the three fundamental sensations. In this manner the fatigued eye is shown to be in a similar physiological state to that of the partially color blind eye. These results are questioned by Edridge-Green (5) who reports that fatigue to yellow obliterates the yellow part of the spectrum without affecting either the red, orange or green.

It is important to note that selective fatigue (according to Burch) involves a loss of brightness, as well as of color, in the part of the test spectrum affected. This loss of brightness is much greater in that part of the spectrum which is nearest the fatiguing light than in other parts. This has been confirmed by Abney (3) using weaker fatiguing lights which were, however, undoubtedly brighter than the test lights. He describes a method of establishing the luminosity curve of the fatigued eye and shows that it corresponds to the luminosity curve of the partially color blind eye (anomalous trichromat).

Allen (6) has recently strengthened this point of view by results obtained with an entirely different method. Taking the critical flicker frequency curve of the spectrum of acetylene light (i.e., the frequency which extinguishes flicker plotted against wave lengths) he shows that this can be selectively modified by fatigue. By fatiguing with red light ($\lambda = 670 \text{ m}\mu$), for instance, he lowers the critical frequency for the spectrum from 620 to the red end without affecting that of other parts. Four points of the spectrum, 420, 470, 570 and $660 \text{ m}\mu$ do not produce this differential fatigue. It is difficult to understand why light at $660 \text{ m}\mu$ does not produce differential fatigue, since at this point only one sensation is evoked to any great degree. Violet light ($420 \text{ m}\mu$) might also be expected to give differential fatigue, at least in relation to green. Unfortunately Allen does not specify the brightness of his fatiguing lights.

Troland (7) is inclined to the view, owing to the symmetry of the visibility curve, that brightness sensitivity is served by a mechanism which is independent of that serving chromatic sensitivity. One of his experimental confirmations of this point of view consists in an investigation of differential brightness fatigue for stimuli of different chromatic value. He found that fatigue to one chroma reduces the brightness stimulus value of all chromas to a nearly equal extent. The brightness of his fatiguing and test fields were nearly equal.

This result is to be expected in the light of Burch's (2), (8) statement that the fatiguing light must be much brighter than the test light in order to decrease either color or brightness in any part of the test spectrum. After a short exposure to a moderately bright light the physiological activity of the retina is automatically adjusted to the stimulus (light adaptation) so that there is no further lowering of the stimulus value of the light (fatigue). Under these circumstances we could hardly expect differential fatigue.

Troland moreover points out very clearly that if the test field is suddenly dimmed, a very strong "brilliance" contrast will appear. This, of course, is *prima facie* evidence of differential fatigue and is the only way in which differential fatigue could be expected to show itself appreciably in terms of the three components theory.

Inasmuch as color blindness is essentially a defect in hue discrimination, and since evidence points to a relation between the effects of selective fatigue and color blindness, partial or complete, it was decided to study the influence of selective fatigue upon the hue discrimination. Our investigations have also brought forth evidence bearing upon the moot question of the relation of "brilliance" and "chroma" in vision.

The ability to discriminate hue has been determined by a number of observers and recently very carefully for the eyes of the authors (1). The threshold of discrimination (see fig. 1 triangles) is lowest in four parts of the spectrum (minima), namely: *a*, in the violet at 437 to 443 $m\mu$ with a value of 1 $m\mu$; *b*, in the blue-green at 484 to 496 $m\mu$ with a value of 0.25 to 1.5 $m\mu$; *c*, in the yellow or yellow-green at 565 to 587 $m\mu$ with a value of 0.5 to 1.0 $m\mu$; and *d*, in the orange at 620 to 630 $m\mu$ with a value of 1.0 to 1.5 $m\mu$. There is considerable variation in the position and value of these points for different individuals and for the same individual under different conditions.

Apparatus. When the test spectrum is of low luminosity, the fatiguing lights need not be very bright in order that "color blindness" should be fairly complete. The fatiguing lights were furnished by a

spectroscope without the eye piece, placed conveniently so that the observer by turning the head could look into the ocular aperture. The arrangements were such that a circular part of the farther face of the prism 4° in diameter was seen illuminated with homogeneous light. The apparatus was calibrated by means of a Hilger wave-length spectrometer so that light of any desired wave-length might be obtained. The field brightness for the various wave-lengths was photometrically determined by direct comparison and calculated in millilamberts as seen through the natural pupil. The test lights were furnished by our second apparatus (1) in which the field size is 3° in diameter.

Procedure: Red fatigue. The fatiguing apparatus was set so that it delivered a fairly narrow band of light centered at $650\text{ m}\mu$ with a field brightness of 85 millilamberts. One of the spectrometers of the hue discrimination apparatus was set at $650\text{ m}\mu$, the other at some shorter wave-length. The fields were then adjusted to equal luminosity (about 1 photon) at which intensity all of the tests were made (except the orange-red fatigue). The eye was then carefully fatigued by fixating the center of the fatiguing field. At the end of 30 seconds the subject as quickly as possible adjusted the brightness of the two test fields to equality by changing the brightness of the shorter wave-length. This was necessary because the luminosity match as made by the normal eye was often not valid for the fatigued eye. In case the subject answered that there was a perceptible difference in hue between the two parts of the field the test was repeated, again after fatigue, so as to rule out the chance that the observed hue difference was due to a partial recovery from fatigue before the luminosity match could be made. This procedure was repeated until a distinct hue difference without brightness difference was perceptible. The spectrum was scaled in this way, the threshold of perceptible difference being found every $10\text{ m}\mu$ unless it had a value greater than $10\text{ m}\mu$. In such a case the next determination was made $20\text{ m}\mu$ further down. The unfatigued eye was occasionally tested to be sure that the brightness of the test lights was sufficient to give values in accord with those previously determined for the normal eye.

The results are shown in figure 1 (curve in solid circles). The points plotted represent the average of the results of two observers, these alone being plotted since there is no significant difference between individual curves and the average. When the red fatigue curve is compared with the normal it is seen that the ability to discriminate hue is practically non-existent from the red to the blue-green at $490\text{ m}\mu$.

The threshold then falls suddenly and closely follows the normal curve as far as $460\text{ m}\mu$, below which it again rises. The normal regions of minimum threshold in the orange and yellow-green are obliterated. These are regions of maximum contrast between the red and green sensations. The practical elimination of the red sensation of course reduces the number of hues that can be differentiated in the orange, yellow and yellow-green, while it does not greatly affect the region of contrast between the blue and green sensations. The shift in the position

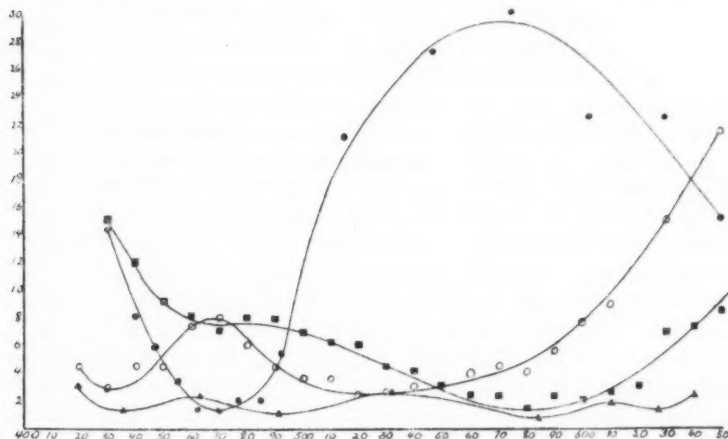


Fig. 1. Solid triangles = normal hue discrimination curve; solid circles = protanopic (red blind) hue discrimination curve produced by fatiguing the eye with red light; open circles = deuteranopic (green blind) curve, produced by fatiguing the eye with green light; and solid squares = tritanopic (blue blind) curve, produced by fatiguing the eye with blue light. In this and the following figure abscissae are wave-lengths in $\text{m}\mu$, ordinates the least perceptible difference (difference threshold) in $\text{m}\mu$.

of the minimum by $20\text{ m}\mu$ toward the blue is not much greater than we found to occur in the same individual under different conditions (1).

The obliteration of the minimum in the violet indicates that it is a region of contrast between the blue and red sensations, i.e., that there is more of the red sensation evoked at $440\text{ m}\mu$ than at $445\text{ m}\mu$ (3), (4).

The red fatigue curve is essentially the same as that of a protanope (1) with the difference that the discrimination in the upper half (above $570\text{ m}\mu$) of the spectrum is somewhat better.

The names given the contrast colors which are just perceptibly different are indicative of the general nature of the retinal changes resulting from the action of the fatiguing light. Wave-length $650\text{ m}\mu$ is called a "light orange"; when contrasted with $630\text{ m}\mu$ it becomes an "unsaturated reddish" against the "green" of $630\text{ m}\mu$. This indicates that the process responsible for the red sensation is still functional. The contrasts between colors from 630 to $550\text{ m}\mu$ are designated "yellowish-green" against "green" by H and as either "orange" against "green" or "brick red" against "blue green" by L. The red sensation would have been completely obliterated if the fatiguing light had been brighter and there would have been no changes in hue due to yellowness in the green part of the spectrum (2). The names given wave lengths in the blue-green where hue discrimination is similar to the normal were the same as for the unfatigued eye, namely, blue, blue-green and green.

In the region of the violet minimum threshold, the contrasted colors were called "blue" and "indigo" by L and "blue" and "darker blue" by H. For H the "redness" which distinguishes violet was absent, while L preferred the term "indigo" to "violet" or "darker blue."

Green fatigue. The eye was similarly fatigued by a green light with a middle wave-length of $517\text{ m}\mu$ and with a field brightness of 52 millilamberts. The spectrum was scaled as above described for red fatigue. The test fields were of the same brightness (1 photon); this brightness was kept constant throughout the test. The average curve is shown in figure 1 open circles. Inspection of it shows that there is a region of minimal threshold in the violet near $430\text{ m}\mu$. This is very near the average normal position of the first minimum. The names given to the contrast colors in this region are the same as the normal, namely, blue and violet.

At the position of the second or blue-green minimum ($480\text{--}500\text{ m}\mu$) the difference threshold, after attaining a very high value at $470\text{ m}\mu$, has begun to fall off again. The contrast colors are called "unsaturated dirty blue" against a "good blue." In the neighborhood of $520\text{ m}\mu$ the curve gradually falls to a minimum similar to that discovered in the normal curve when the spectrum is scaled from blue to red (1). The colors are here described as "pink" against "bluish gray." The difference threshold then gradually rises to the red end of the spectrum. There is no sign of either the third, yellow, or fourth, orange, minimum. The contrast colors are called "unsaturated red" against "bluish gray" as far as 560 or $570\text{ m}\mu$; above this "brown" or "red" against "purplish gray." A fact worthy of remark is that there seems to be a

bluish component in the sensation as far as 630 $m\mu$. This is brought out only through the combination of green fatigue and simultaneous contrast. It is not seen even with the fatigued eye above 570 $m\mu$ when only one wave-length is presented in the test apparatus at a time.

In comparing this curve with that of deuteranopes (9) it is seen that it is similar in having a region of minimal threshold in the green although this point is about 20 $m\mu$ higher in the spectrum than it is in the curve of the "green blind" eye. This is a small discrepancy when we consider such factors as retinal pigmentation, and the probability that the fatigue was both incomplete and imperfectly selective; the latter because both the red and the blue sensations are present at 517 $m\mu$. Whether a violet minimum is really present in the hue discrimination of a deuteranope should be investigated.

The shape of the green fatigue curve is explicable in terms of a deficiency of the green sensation, assuming that it is represented throughout the spectrum in the proportions shown in the standard sensation curves. The region of minimal threshold (near 520 $m\mu$) is due to the fact that near here the red and blue sensations are in greatest contrast to each other (3), (1), and the removal of the green sensation allows this contrast to become visible. The high value of the threshold in the blue, green and yellow is due to the lack of the green sensation with the consequent absence of contrast to blue or red sensations.

The Hering curves, which have no exact experimental justification (10), are quite inapplicable to the present situation. There is no unique relationship between the red and green sensations. Green fatigue affects the distribution of blueness throughout the spectrum quite as much as it does the distribution of redness as seen in the test lights.

Blue fatigue. Fatiguing fields of two wave lengths (460 $m\mu$ and 440 $m\mu$, field brightness of 3 millilamberts) were used and gave such similar results that they may be discussed together. The procedure was the same as in the case of red and green fatigue. But since the brightness of the fatiguing light was less, the fatigue, though selective, was not complete enough to entirely eliminate the blue sensation from the comparison lights.

An inspection of the curve (fig. 1 solid squares) shows that while the fourth or orange minimum is absent, the third, or yellow-green, minimum is practically normal. The absence of the orange minimum is attributed to general fatigue rather than to any selective action of the blue light, because it is lacking in all of our fatigue curves, even from that obtained after fatiguing with white light. There is very little of

the green sensation in the region of the orange minimum, so that general fatigue, affecting the three sensations equally, might reduce the green sensation below visibility without markedly affecting the red sensation. The orange minimum is frequently absent in normal curves (1).

The names given to wave-lengths in the region of the yellow-green minimum are the same as for the normal eye, except that there appears to be a slight relative lessening of the green sensation, especially when the eye was fatigued with light of wave-length $460\text{ m}\mu$.

The curve from $580\text{ m}\mu$ rises to the violet end of the spectrum. There is no indication of the normal second, or blue-green minimum. The contrast colors as seen between 580 to $510\text{ m}\mu$ are "orange" or "yellow-green" against "green." Below $500\text{ m}\mu$ they are "blue-green" against "bluish gray." The contrasts from 480 to $460\text{ m}\mu$ were called "green" against "blue-green," and from 460 to $430\text{ m}\mu$ "unsaturated blue" against "violet" or "reddish-violet." The blue sensation was not completely eliminated, as stated above, and the violet, consisting of the red and the blue sensation, as there is some evidence that it does (3), (4), has a good "background" against which to show.

It would be very interesting to compare this curve with the hue discrimination curve of a tritanope (blue blind). So far as we know, such a curve has not been established. Steindler (9), calculating from the ordinates of the three sensation curves, has estimated that minima will be found to occur at $620\text{ m}\mu$ and $540\text{ m}\mu$, with a smaller one at 480 to $490\text{ m}\mu$. The violet minimum should undoubtedly be absent, because of the shortening of the blue end of the spectrum. The blue-green minimum would of course be eliminated and in its stead there might appear a region of lowered threshold near $500\text{ m}\mu$, due to the contrast of the lower end of the red sensation curve with the green. On the basis of König's (11) curves there is no apparent reason for a shift in the position of the third and fourth minima from their normal places.

The effect of selective fatigue on hue discrimination in a protanope. In an earlier paper (1) we have figured the hue discrimination curve of a protanope. The region of minimal threshold was found to be near $495\text{ m}\mu$, with a value of $1.5\text{ m}\mu$, from which point the curve rose rather sharply in both directions. There was no secondary minimum in the yellow as Steindler (9) has figured.

When the eye of this subject was fatigued with light of $\lambda\ 570\text{ m}\mu$ using the same intensities and field sizes as on the normal eye, the number of discernible hue contrasts in the entire spectrum was reduced to three. Thus selective fatigue rendered him practically a "monochromat" (but not an "achromat").

The contrast colors visible to him are as follows: red end of spectrum to 500 $m\mu$, "reddish" against "bluish;" 500 to 470 $m\mu$, "reddish" against "bluish;" 470 $m\mu$ to the blue end of the spectrum, "bluish" against "purple." He was not sure whether the fatiguing light was "red" or "green."

The experiment was repeated with a fatiguing light of λ 440 $m\mu$ with practically the same results. Four contrasts were found, namely: violet end to 450 $m\mu$; 450 to 480 $m\mu$; 480 to 510 $m\mu$; and 510 $m\mu$ to long end of spectrum. It is important to notice that light of λ 570 $m\mu$ which induces non-selective fatigue in the normal eye (vide infra) has a decidedly selective effect upon the "red blind" eye.

Non-selective fatigue. In contrast to the above experiments, it is of interest to note the effect on hue discrimination of fatiguing the eye with white, orange-red, yellow and blue-green light. The white light was obtained by diffusing the light from an ordinary 100 watt gas-filled tungsten lamp through ground glass. The lamp was about 50 cm. from the screen. The field size was the same as in the previous experiments. The orange-red, 630 $m\mu$, field brightness, 156 millilamberts; yellow, 570 $m\mu$, field brightness, 342 millilamberts; and the blue-green, 480 $m\mu$, field brightness 9 millilamberts, were obtained by means of the apparatus above referred to. The brightness of the test fields was the same as in the previous experiments, except in the case of the orange-red, which was somewhat brighter.

The hue discrimination curves are shown in figure 2. They do not differ markedly from the normal except that the average threshold is higher and the orange minimum is lost in all, and the violet minimum in some. The color names are practically normal, though the fields appear less saturated than to the unfatigued eye, especially in the parts of the spectrum of similar, or nearly similar wave-length to that of the fatiguing light. With blue-green and yellow fatigue there is evidence from the color names that the green sensation has been selectively fatigued to some extent. The same is true in the blue-green region in case of yellow fatigue, and in the yellow region in the case of blue-green fatigue. This slight selective fatigue does not affect the position of the minima. The orange-red fatiguing light (630 $m\mu$) exerted a similar selective influence upon the color names involving the red sensation.

The striking similarity between hue discrimination in an eye that has been fatigued by orange-red, yellow, blue-green and white light, and the general similarity between this and normal hue discrimination indicates that the effect here is non-selective. It is in striking

contrast to the result of fatiguing the eye with red, green or blue (violet) lights. The physiological basis of this difference is undoubtedly that as a result of non-selective fatigue two, or three in case of white, sensations are being fatigued equally, and by light which is not (in case of yellow or blue-green) able to excite them maximally. The effect is that their relationships remain the same. As a result of selective fatigue one of the sensations is fatigued to such an extent that it functions little or not at all in response to the relatively weak stimuli of the test fields, while the other sensations are relatively unaffected by the fatigue. As a consequence only one sensation is markedly active in spectral regions where normally there are decided contrasts between two sensations.

These results are of especial interest to those investigators who hold, in accordance with the Hering theory, that yellow is served by a single

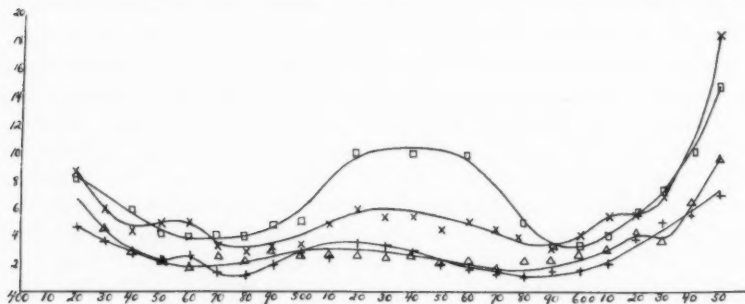


Fig. 2. Hue discrimination curves showing the effect of orange-red (crosses), yellow (squares), blue-green (plus signs), and white (triangles) fatigue.

receptor process. Our findings, on the contrary indicate that at least as far as the receptor processes go, yellow, blue-green and white must be classed together. Orange-red and blue-green appear in consciousness as compound or mixed sensations, while yellow and white are regarded by most psychologists as simple, unmixed sensations. We are forced to give up the hypothesis, at least in case of yellow, that this subjective unity depends upon the functioning of a single "yellow" receptor. We shall turn now to evidence bearing upon the physiological nature of the receptors serving the white or "brilliance" sensation. Is there a separate white receptor or is "whiteness" due to a summation of the effects of stimulating the three chromatic receptors?

The effects of selective fatigue on luminosity, with special reference to the relation of "brilliance" and "chroma" vision. It has been shown

that selective fatigue with red, green and blue light wipes out the chromatic aspects of the red, green and blue sensations, when these are excited by a stimulus that is not as intense as the fatiguing stimulus. It has been further shown that fatiguing the retina with orange-red, yellow, blue-green or white light does not have any such specific effect.

In the course of our preliminary observations it was found that if the test field was approximately of the same order of brightness as the fatiguing field, the fatigue was non-selective in character and that hue discrimination was relatively unaffected. In other words, a bright red field, for instance, appeared red after red fatigue but a dim red field lost its redness (cf. 8).

Troland (7) has shown, when the test field (e.g., all red) is of the same brightness as the fatiguing field (half red and half green), that there is relatively little difference between the "brilliance" response of the retina corresponding to the two parts of this field, in spite of the fact that one part of the retina has been fatigued by red light while the other part has been fatigued by green light. (See also Tufts (12)).

This is what might be expected in terms of the Young-Helmholtz theory. There is no *a priori* reason for expecting the "brilliance" aspect of the red sensation to be more quickly fatigued than the chromatic aspect and the chromatic value of a moderately bright stimulus is relatively unchanged by continued fixation. It would be a simple matter to arrange the relative brightness of our test and fatiguing fields so as to actually induce fatigue, for instance, of the red sensation, so that its redness disappears, or is very much reduced, in the test field. We would then be in a position by the proper photometric measurements to prove or disprove the theory that "brilliance" is independent of "chroma."

Using a fatiguing light of known and constant brightness we studied its effects upon the relative brightness of a pair of dim test lights one of which was of the same wave-length as the fatiguing light, and the other of a totally different color. The test lights were then increased in brightness and the test repeated with the same fatiguing light.

In general terms we found that blue fatigue has but little effect upon the brightness of the blue test light as compared with that of a green or red test light. One of us (L) saw no change in their relative brightness, while the other (H) saw a slight but constant relative dimming of the blue as a result of blue fatigue. The differential effect of green fatigue was much more pronounced and that of red very marked indeed. When the brightness of the test lights, the one red and the other blue or green,

was such that they appeared rather dim, but of equal brightness to the normal eye, the red field was absolutely black to an eye fatigued with red, while the blue or green field, though somewhat dimmed was of normal color and fairly bright. This type of differential fatigue was found to decrease as the test lights were brightened, and to disappear entirely with relatively bright test fields.

Our method of recording the degree of differential fatigue was as follows: With the unfatigued eye the two fields were matched as to

TABLE 1

FATIGUING LIGHT		λ OF TEST FIELDS	INTENSITY OF TEST FIELDS TO UNFATIGUED EYE	PERCENTAGE INCREASE OF HOMOCROMATIC STIMULUS TO MATCH HETEROCROMATIC STIMULUS AS SEEN BY FATIGUED EYE	COLORS AS SEEN BY FATIGUED EYE
λ	Intensity				
L.					
<i>mμ</i>	<i>millilamberts</i>		<i>photons</i>		
650	85	650-460	0.07	1730	Grayish (green): blue
			0.77	248	Yellow-green-gray: blue
			7.5	0	Deep orange-red: blue
		650-517	0.07	1730-2340	Black: green (gray green)
			0.77	248- 366	Pink-green
			7.5	37- 63	Red-green
H.					
650	85	650-460	0.07	1730-2000	Black: blue
			0.77	291	Almost black: blue
			7.04	30	Orange-red: blue
			18.0	0	Red: blue
		650-517	0.07	1009-1730	Almost black: green
			0.77	450- 535	
			10.75	80- 84	
			20.0	0	

brightness. This could be done with a fair degree of constancy because of much practice on our part. The eye was then fatigued for one minute with light of the same wave-length as that of one of the fields, and the test fields rematched as to brightness by the fatigued eye. The amount of change was recorded and the relative brightness changes calculated from nicol readings. The values are given in the tables below.

Table 1 shows that to match a weak blue or green field with a red field, the red fatigued eye requires seventeen to twenty-three times as

much red light as the normal eye. If the test fields are brighter, the red fatigued eye required a smaller increase (240 to 360 per cent) of red light to make the match, and if the test fields are quite bright the match seems as good to the red fatigued eye as to the normal eye. Comparison of the last two columns in the table shows that as seen by the fatigued eye, the "redness" and the relative brightness of the 650 $m\mu$ field vary together. When the fatigue lessens the relative brightness of

TABLE 2

FATIGUING LIGHT		λ OF TEST FIELDS	INTENSITY OF TEST FIELDS TO UNFATIGUED EYE	PERCENTAGE INCREASE OF HOMOCHROMATIC STIMULUS TO MATCH HETEROCHROMATIC STIMULUS AS SEEN BY FATIGUED EYE	COLORS
λ	Intensity				
L.					
<i>mμ</i>	<i>millimiberts</i>		<i>photons</i>		
517	52	517-460	0.175	200	
			0.4	110	
			0.7	0	
		517-650	0.175	207	
			0.4	103	
			0.7	0	
H.					
517	52	517-460	0.068	376	Black: blue
			0.175	195	
			0.4	110	
			0.8	0	
		517-650	0.35	251	Gray: blue
			0.65	53	
			2.46	0	
440	Very slight differential fatigue				
460	Very slight differential fatigue				

the homochromatic (in this case red) field, it lessens its chromatic value to a similar extent. When the fields are rematched, the chroma of the homochromatic field returns.

The differential brightness change is much less in case of green fatigue, (table 2) and scarcely observable in case of blue or violet fatigue. In this connection it is to be noticed that, according to Abney (3), the areas of the red, green and blue sensation curves when plotted with relation

to luminosity are in the ratio 527 : 248 : 3.26 respectively. The effect of a very bright blue fatiguing field was not ascertained.

When the eye is fatigued by yellow light (table 3), in spite of the fact that the fatiguing field is about three times as bright, the relative decrease in brightness is not at all comparable with the relative decrease in brightness in case of red fatigue. The results are similar quantitatively to those of green fatigue, again in spite of the fact that the yellow

TABLE 3

FATIGUING LIGHT		λ OF TEST FIELDS	INTENSITY OF TEST FIELDS	PERCENTAGE INCREASE OF HOMOCHROMATIC STIMULUS TO MATCH HETEROCHROMATIC STIMULUS AS SEEN BY FATIGUED EYE	COLORS
λ	Intensity				
L.					
mμ	millilamberts		photons		
570	342	570-460	0.33	289	
			0.63	66	
			3.06	53	
			4.68	0	
		570-517	0.33	300	
			0.63	70	
			4.06	30	
		570-650	0.33	290-300	
			0.63	69	
			6.37	18	
H.					
570	342	570-517	0.60	65	
		-650	0.63	0	
		-460	—*	—*	
480	Practically no differential fatigue				

* Not tried.

fatiguing light is much brighter than the green. We are unable to confirm Allen's (6) finding that yellow light (570 mμ) does not cause differential brightness fatigue.

The effect on the relative brightness of homo- and heterochromatic lights of fatiguing the eye to blue-green was uncertain. The brightness available was insufficient to markedly change the chromatic value or brightness of the test fields.

These experiments were repeated on the dichromatic eye. It was found that, whereas fatigue with violet ($440\text{ m}\mu$) did not give rise to any appreciable differential brightness fatigue, fatigue with yellow ($570\text{ m}\mu$, brightness of 342 millilamberts to the normal eye) showed a marked differential effect when the test fields ($517\text{ m}\mu$ and $460\text{ m}\mu$) were fairly dim. When their brightness was increased this effect, as in the case of the normal eye, was gradually lost.

It is to be emphasized that in addition to the differential brightness fatigue which has just been discussed, there is also a general fatigue which affects all colors in the spectrum, no matter with what light the eye had previously been fatigued. For example, after red fatigue a red-blue test field would appear much dimmer to the fatigued eye than to the normal eye. To the normal eye the red and blue parts of the field would appear equally luminous, whereas to the fatigued eye the red field would appear much the dimmer. It is evident that this phenomenon must be referred to some process that affects all three of the receptors, such as light adaptation. The suggestion that the rods may be involved deserves consideration.

SUMMARY

1. The hue discrimination curves of an eye fatigued by strong homogeneous red and green light are similar to those typical of the protanope (red blind) and deuteranope (green blind) respectively.

2. Fatiguing the eye by strong homogeneous blue (or violet) light changes its hue discrimination so that it is similar to that calculated from the sensation curves of the blue blind (tritanopic eye).

3. Fatiguing the eye by strong homogeneous orange-red, yellow, blue-green or by white light, has no selective effect upon hue discrimination. This indicates that the yellow is not served by a unitary receptor process as is postulated by the Hering theory of color vision. Our experiments supply no evidence that there is an antagonistic relationship between any receptor pairs.

4. Brightness is not separable from chroma. Fatigue reduces these two aspects of a chromatic stimulus together.

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TEMPORAL VARIATION IN THE FUNCTION OF THE GYRUS PRECENTRALIS IN PRIMATES

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Received for publication May 21, 1923

In much of the earlier work upon the effects of electrical stimulation of the cerebrum the stimuable points were looked upon as the physiological expression of precisely localized structures having rather simple anatomical connections with lower motor neurones. The points were regarded as absolutely stable and maps of areas in different species were published with the conviction that they represented definite anatomical differences having evolutionary significance.

Recent evidence has tended to discredit this view of precise anatomical localization. Franz (3) mapped the arm and leg areas in a number of rhesus monkeys and found little correspondence in localization of stimuable points among different individuals. Even the two hemispheres of the same brain showed divergence in the localization of points exciting the same movements. This result led Franz to conclude that the apparent localization revealed by electrical stimulation is largely the result of physiological rather than strict anatomical conditions. In summing up the work he says (page 147):

The results of the present research, in conjunction with the data of others which have been recorded above, indicate that the connections which are made by way of the cortical motor cells are not definite in the sense, for example, that there is a passage of an impulse from a Betz cell in the anatomically defined cerebral motor region to another particular efferent cell in the spinal cord, but that the connection is, in special senses of the terms, promiscuous or irregular. By these last terms I mean only that the connections which one particular efferent or afferent cell makes are connections with a great number of neurones, and that the impulses resulting from the activity of a cell body may affect many other cells.

Quite similar results were obtained by Stout (6) in studies of the motor area of the cat. This author seems to imply a functional cor-

relation of the extent of the stimuable areas with the habit systems of the individual when he says (page 208 ff.):

The great number of points producing reactions of certain kinds and the wide distribution of such points on the cerebral surface probably indicate the frequent demand for that particular type of movement in the daily life of the individual and the frequent combination of such types of movement in the production of certain forms of activity, which are in constant demand in the individual's reaction to his complex environment.

This work demonstrates the existence of extensive individual variations in the localization of function of the stimuable cortex. There is also evidence for some variation in function under different conditions of facilitation and inhibition. Brown and Sherrington (2) found that a point which on one stimulation gave contraction might later give extension of the same muscle group. Such a reversal of movement might result after repeated stimulation of the point, after stimulation of an antagonistic point, after epileptoid discharge, or after stimulation of the afferent nerve of the contralateral limb. They mention one case of seeming spontaneous reversal of effect when a point was re-examined 28 hours after a first test. Their study was restricted to pairs of antagonistic muscles and hence offered little chance of discovering more extensive alterations in the movements elicited from the points studied.

In a series of papers (1) Brown has extended this work and has done much to clear up the mutual relations of points within the central convolutions.

Leyton and Sherrington (5) described a number of cases where successive stimulations of a cortical point resulted in dissimilar movements, after intervening stimulation of other areas. Some of these "deviations of response" involved changes as great as that from finger to shoulder movements and even overlapping of the face and arm areas. The authors regard these deviations as temporary and ascribe them to facilitation from other areas stimulated during the course of the experiment, acting either upon the cortical or subcortical centers.

This conception seems to imply the belief that there is a primary movement typical of each cortical point, stable over long periods of time, which may be modified by facilitation but will return spontaneously to its primary condition when the effects of facilitation have worn off. In several experiments the authors stimulated the same area at intervals of from a few days to several months. They report that the same movements were obtained in the different tests. These reports are

fragmentary, however, and do not include any systematic exploration of extensive areas with a view to determining temporal variation. It is possible that, within the limits of deviation found in the experiments, no one movement or pattern of movements is more characteristic of a given cortical point than another and that the movements elicited at any given time are dependent upon the previous condition of activity of the cortex, rather than upon any innate primary function of that point.

Such studies indicate that the functional localization of the motor cortex is capable of wide variations under the influence of the activity of other parts of the cerebrum. They leave the question of precise anatomical localization undecided, however. It is possible that each cortical point is capable of calling out a primary reaction which is relatively stable, although capable of temporary modification: it is possible that the reaction elicited from each motor point is wholly dependent upon previous conditions of stimulations and that apparent anatomical localization within the motor area arises only through the relative constancy of dynamic conditions within the time limits of the experiment: or the truth may lie somewhere between these extremes, in a gross anatomical localization within which the finer movements are determined by transient physiological conditions.

The question can be answered in part by exploring and mapping the same stimulable area at different times, allowing a sufficient interval for a change in the "set" of the organism between successive tests.¹

Stability of cortical points under such conditions would argue for a definite anatomical basis of function. Great variation in localization on the contrary would indicate that unstable physiological conditions underlie a merely transitory localization. The present paper is a report of a series of tests of localization in the gyrus precentralis made at intervals of from one to fifteen days to test the extent of temporal variation.

Methods. Under deep chloroform-ether anesthesia the skull of a small rhesus monkey was trephined and the opening enlarged with bone forceps until the greater part of the right precentral gyrus was exposed. The dura was trimmed away, the other meninges left intact. The distances separating the chief visible landmarks on the exposed surface were measured and a map, enlarged to twice the size, was laid off with proportional dividers. The area exposed is indicated by the

¹ The desirability of extending studies of variation in the motor cortex to its temporal aspects was pointed out to me some years ago by Dr. S. I. Franz, to whom I wish to acknowledge my indebtedness for the suggestion.

dotted line in figure 1. This is a camera drawing of the brain, after hardening in 10 per cent formalin, upon which the original map was projected. The inner margin of the area first exposed paralleled the longitudinal fissure at a distance of 5 mm. Its lateral margin passed 6 mm. from the fissure of Sylvius. Caudad it included a part of the post-central gyrus. The area was explored and the excitable points indicated on the map. After exploration, which was performed under aseptic conditions, the exposed area was covered with a thin flexible sheet of mica to prevent adhesions, the skin flaps closed with inter-

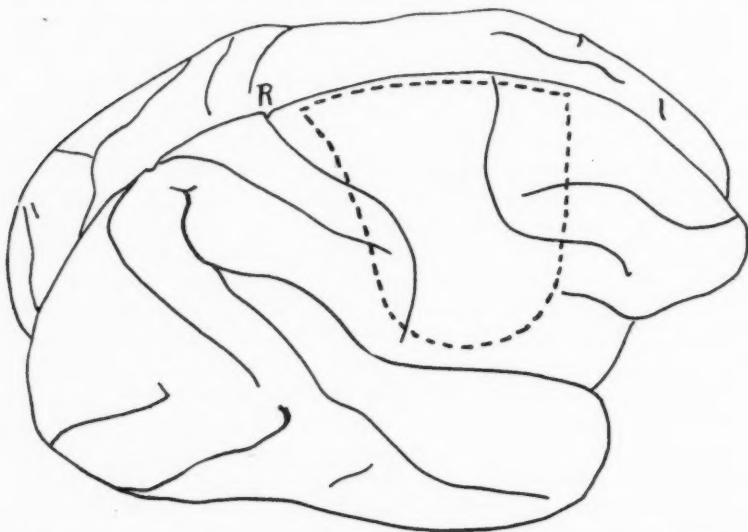


Fig. 1. Camera outline of brain from right dorso-lateral aspect. The extent of the area mapped is shown by the broken line. $\times \frac{1}{3}$.

rupted sutures and securely bandaged. The exploration was repeated in four consecutive tests at intervals of from one to fifteen days.

Stimulation was given by the bipolar method, the points of the electrodes separated by 1 mm. At the beginning of each experiment the threshold was determined for the point *b4* (fig. 2) and current intensity was maintained just above this limen except where areas proved relatively inexcitable. When such areas were encountered the current intensity was increased until responses appeared or until there was indication of spread of current to involve neighboring areas.

The electrodes were applied at each stimulation for less than one second so that in most cases only the primary movements of the point were elicited.

The points stimulated were located by reference to visible landmarks on the surface of the cortex and by measurements with proportional dividers, particularly from the two median angles of the skull opening. The points tested were separated by approximately $2\frac{1}{2}$ mm. The method of measurement assures, I believe, the identification of the points in successive trials. It is possible that adjacent points were sometimes confused in different tests, but the possibility of such a defect in technique is inadequate to account for the wide variation found in many cases.

In order to avoid differences in facilitation or deviation in the sense of Leyton and Sherrington the same order of stimulation was followed in successive tests. Exploration was begun at the postero-median corner of the field and continued in the order in which the points are listed in table 1. After systematic exploration in this manner, the chief points from which distinct movements had been obtained were again stimulated in irregular order to verify the first findings and to test the possible influence of the sequence of stimulation upon the character of the movements obtained. In general these check stimulations consistently verified the findings of the systematic exploration. In a few cases deviations of response were noted which were clearly the result of the stimulation of the face area at the end of the systematic exploration, but the major differences in the findings of successive tests were verified in each case and proved stable for the duration of each test.

During the tests the cortex was kept moist with sponges wrung out from warm physiological saline. The electrodes were applied by the experimenter who also recorded the position of the points stimulated upon the enlarged map. Movements were observed and recorded by an assistant.²

An effort was made to obtain the same degree of narcosis in the different tests, but this was scarcely possible, owing to marked differences in the resistance of the animal to the anesthetic at different times.

CONDITIONS OF THE DIFFERENT TESTS. *Test I.* The first exploration was made on March 27th. The movements elicited from the excitable area are given in table 1. The field seemed to include a considerable portion of the leg area, and points for all segments of the arm. The lateral margin of the field, later giving movements of the face, was inexcitable to any strength of current. The postcentral gyrus remained inexcitable in

² I am indebted to Mr. Lester Wiley and to Mr. Carney Landis for assistance in this work.

all the tests. After exploration the wound was closed and the animal returned to his cage. On recovery from the anesthetic he showed no symptoms of motor disturbance.

Test II. Twenty-four hours later, March 28th, the animal was again anesthetized, the cortex exposed and mapped as before. The movements elicited in this test are given in table 1, column 3. The threshold was slightly higher (one-half division on the Porter inductorium) but epileptoid contractions were occasionally elicited on very slight stimulation. The opening was again closed and the animal kept under observation for 16 days. During this time there was no indication of any motor disturbance and healing of the wound progressed rapidly.

Test III. On April 13th the cortex was again exposed. It appeared perfectly normal, without adhesions, and it was mapped as before. The threshold was approximately that of the first test. The movements obtained are given in table 1, column 4. The wound was closed and the animal kept under observation. No motor disturbances were noted until April 25th. On April 23rd the animal tore off his bandage and infected the wound. Two days later a very slight paresis of the left arm developed. It was detectable only in an occasional extension of the arm when the animal was at rest. In feeding and locomotion the arm was used in normal manner. I had intended to test the effects of disuse, but the infection made it necessary to terminate the experiment. The following test of fatigue was therefore undertaken.

Test IV. Effects of fatigue. April 25th, the monkey was driven about the animal room as rapidly as possible and kept in active motion for 30 minutes. At the end of this time he seemed completely exhausted, lay prone on the floor and submitted to every indignity without making an effort to rise. He was then quickly anesthetized and the wound opened. Since the last test, a thin membrane had developed over the surface of the exposed cortex. Removal of this entailed superficial injuries to the gyrus, especially over the points *a*, *b*, *c*, *6*, *7*, and *8*, figure 2. The movements elicited by stimulation are given in table 1, column 5. The threshold was found to be slightly higher than in any previous test. This may have been due to the fatigue, to the infection, or to the injuries to the cortex. Had the experiment shown decreased activity the infection would have rendered it meaningless, but the variety of movements elicited seems significant in spite of the defects of technique.

At the end of this test the wound was again closed. On the following day the animal showed a well-developed paresis of the left limbs.

Examination of the wound showed an inflammation of the exposed cortex and a slight hernia. The area was wholly inexcitable to faradic stimulation although stimulation of the white fibers still induced movements. Enlargement of the skull opening and exploration of the surrounding areas was begun. Movements of the leg were obtained from the area medially to the opening, but with further enlargement of the opening there was a sudden disappearance of all excitability, involving both the cortex and the underlying fibers. This depression persisted for half an hour, at the end of which the experiment had to be terminated. During this time the other hemisphere remained normally excitable. It was thus impossible to map the area surrounding the first operative field.

ANALYSIS OF RESULTS. The points stimulated are indicated in figure 2. Each lay within one of the squares marked off by the ordinates and abscissae on the map. The movements elicited from the region of the precentral gyrus in the four successive tests are listed in table 1. The points stimulated are indicated by numerals and letters which give the intersection of ordinates and abscissae in figure 2. In every test the order of stimulation of points was the same as the order in which they are listed in the table. The duration of each test was approximately half an hour, including frequent brief intervals of rest. The movements listed are practically all "primary," that is, the first (and usually only) movement elicited by just supraliminal current applied for not more than one second.

The majority of the reactions obtained from the exposed area were of the arm segment. A relatively large area giving facial movements was exposed but these movements lacked variety and were sometimes completely absent. In the first test an extensive leg area seemed included in the field, but in later tests movements of this segment were rarely obtained.

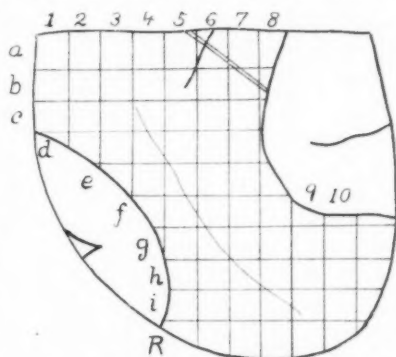


Fig. 2. Diagram of points stimulated on the right precentral gyrus. R = Fissure of Rolando. Points outside of the cross-ruled area were inexcitable.

TABLE 1
List of points explored in the area of the precentral gyrus shown in figures 1 and 2. The first column gives the location of points in figure 2. The remaining columns from left to right list the movements elicited from each point in the successive tests. Primary movements elicited in the systematic exploration alone are given.

AREA	TEST OF MARCH 27	TEST OF MARCH 28	TEST OF APRIL 13	TEST OF APRIL 25 (FATIGUE)
a1	Knee flexed	Leg straightened (insensitive)	Upper arm abducted*	Arm retracted
2	Knee flexed	Back flexed	Arm and shoulder raised	Arm retracted
3	Knee flexed	Arm abducted	Arm and shoulder raised	Arm retracted
4	Arm adducted	Arm abducted	Arm adducted	Arm retracted
5	Arm adducted	Upper arm rotated inward	Arm adducted, elbow flexed, dorsal flexion of wrist	Silent
6	Silent	Upper arm rotated inward	Arm adducted, elbow flexed, dorsal flexion of wrist	Silent
7	Silent	Upper arm adducted, forearm rotated outward	Arm adducted, elbow flexed, dorsal flexion of wrist	Silent
8	Silent	Upper arm adducted, forearm rotated outward	Arm adducted, elbow flexed, dorsal flexion of wrist	Silent
b1	Toes flexed	Leg straightened (insensitive)	Upper arm rotated inward	Arm retracted, forearm rotated inward
2	Knee flexed	Back flexed, arm abducted	Upper arm rotated inward	Arm retracted, forearm rotated inward
3	Knee flexed	Arm abducted	Upper arm rotated inward, elbow flexed	Arm retracted, forearm rotated inward
4	Knee flexed	Arm abducted	Dorsal flexion of wrist	Slight flexion of hip

* On first stimulation a slight flexion of the hip was obtained. Repeated stimulation of the same point later induced only arm movements.

5	Forearm rotated inward	Arm abducted	Forearm rotated inward	Forearm rotated inward	Forearm rotated inward
6	Silent	Upper arm rotated inward	Forearm rotated inward	Forearm rotated inward	Arm abducted
7	Wrist extended	Forearm rotated outward, arm adducted	Forearm rotated inward	Forearm rotated inward	Arm abducted
8	Wrist extended	Forearm rotated outward, arm adducted	Forearm rotated inward	Forearm rotated inward	Head rotated to opposite side, ear raised
e1	Silent	Arm retracted	Dorsal flexion of wrist	Dorsal flexion of wrist	Forearm rotated inward
2	Silent	Arm retracted	Dorsal flexion of wrist	Dorsal flexion of wrist	Fingers flexed
3	Forearm rotated inward	Arm retracted, elbow flexed	Arm adducted, hand closed	Arm adducted, hand closed	Forearm rotated inward, fingers flexed
4	Forearm rotated inward	Elbow flexed	Arm adducted, hand closed	Arm adducted, hand closed	Lateral flexion of wrist
5	Forearm rotated inward	Elbow flexed	Hand closed	Hand closed	Silent
6	Silent	Upper arm rotated inward	Hand closed, arm adducted, elbow flexed	Hand closed, arm adducted, elbow flexed	Silent
7	Silent	Forearm rotated outward	Arm retracted	Arm retracted	Head turned opposite side
d2	Arm abducted	Arm retracted	Fingers flexed, elbow flexed	Fingers flexed, elbow flexed	Forearm rotated
3	Arm abducted	Hand opened and closed	Fingers flexed, elbow flexed	Fingers flexed, elbow flexed	Lateral flexion of wrist, hand closed
4	Arm abducted	Hand opened and closed	Fingers flexed, elbow flexed	Fingers flexed, elbow flexed	Dorsal flexion of wrist, hand closed
5	Arm abducted	Elbow flexed	Fingers flexed, elbow flexed	Fingers flexed, elbow flexed	Dorsal flexion of wrist, hand closed
6	Arm adducted	Arm adducted	Fingers flexed, elbow flexed, shoulder raised	Fingers flexed, elbow flexed, shoulder raised	Head turned
7	Arm adducted	Upper arm rotated inward	Fingers flexed, elbow flexed, shoulder raised	Fingers flexed, elbow flexed, shoulder raised	Head turned, ear raised, eyes turned right

TABLE 1—*Conclude 4*

AREA	TEST OF MARCH 27	TEST OF MARCH 28	TEST OF APRIL 13	TEST OF APRIL 25 (FATIGUE)
e3	Arm abducted	Hand opened and closed	Fingers flexed, elbow flexed	Thumb adducted
4	Wrist extended	Arm retracted	Fingers flexed	Fingers flexed
5	Fingers flexed (strong)	Silent	Fingers flexed	Fingers flexed
6	Elbow flexed	Silent	Fingers flexed	Silent
7	Arm adducted	Arm adducted	Fingers flexed, shoulder raised	Head and eyes rotated
f4	Wrist extended	Arm retracted, hand opened and closed	Fingers flexed, elbow flexed	Silent
5	Fingers flexed	Arm retracted, hand opened and closed	Fingers flexed	Silent
6	Silent	Silent	Silent	Silent
7	Silent	Silent	Silent	Silent
8	Silent	Silent	Silent	Silent
g5	Wrist extended	Arm retracted	Shoulder raised, skin twitched	Silent
6	Thumb adducted	Arm retracted	Shoulder raised, skin twitched, arm rotated inward	Lips pursed
7	Elbow flexed	Lips pursed	Shoulder raised, skin twitched, arm rotated inward	Lips pursed
8	Silent	Lips pursed	Silent	Lips pursed
9	Silent	Lips pursed	Silent	Lips pursed
10	Silent	Lips pursed	Silent	Lips pursed
h5	Wrist extended	Arm retracted	Dorsal flexion of neck	Lips pursed
6 ** etc.	Silent	Lips pursed	Lips retracted	Lips pursed
x5 ** etc.	Silent	Lips pursed	Lips retracted	Lips pursed

** Rows h and i gave same movements throughout in each trial, with the exceptions indicated.

Comparisons of the results obtained in different tests seem to justify the following generalizations.

1. *In each test the movements obtained were quite constant.* After the systematic exploration, which reviewed the points in the order listed, two or three points in each row were again stimulated and in each test the movements obtained in the systematic exploration again appeared. These check stimulations were given in irregular sequence so that it seems probable that facilitation and deviation played little part in determining the reactions listed.

2. *In the tests on different days there was almost no constancy of reaction from day to day.*

a. *In the different tests stimulation of the same point usually gave rise to different movements.* Fifty-seven points were examined. Among these the following proportions of duplicate and diverse movements were obtained.

Number of points which gave a different primary movement at every test.....	22
Number of points which gave the same movement in 2 trials only ..	26
Number of points which gave the same movement in 3 tests.....	6
Number of points which gave the same movement in 4 tests.....	0

Three points were silent throughout the tests. In the above figures only movements were considered. In some cases (a 6-8; c 6; e 6:) the areas were silent in two tests and gave diverse movements in the others. These five cases are included in the group of 22 above.

The 26 points from which the same movement was obtained in two trials include ten cases of duplicate movements of the lips. The mouth area is very extensive and gives little variety of movement so that this duplication receives undue weight when considered in this way. Disregarding the lip movements and silent areas, 50 per cent of the points examined failed to give duplicate movements in any two tests.

To determine whether mere reversal of movement as described by Brown and Sherrington (2) is responsible for the apparent variation of function or whether the variation is more extreme, we may compare the movements elicited from the same point at different times.

b. *The results show that not only does reversal of function occur but the same area may show, as primary movements, flexions or extensions of different joints or even body segments.* Movements elicited from the arm area are frequently restricted to a single joint or segment of the arm. Rarely more than two such segments are involved in the reaction. Of

the 57 points tested 29 gave movements restricted to different joints at different times, as point *b7* which gave movements of wrist, forearm and shoulder only in three different tests. Figure 3 shows in stippling the points which gave exclusive movements of different segments of the arm in different tests, with no duplication of movements in at least two tests.

Further, the variations of function extend even to different bodily segments. Five points gave only movements of the leg in one test and only movements of the arm in the others: five gave neck and eyes in one test and only arm in others: three gave face only and arm only in different tests. The location of these areas is given in figure 4.

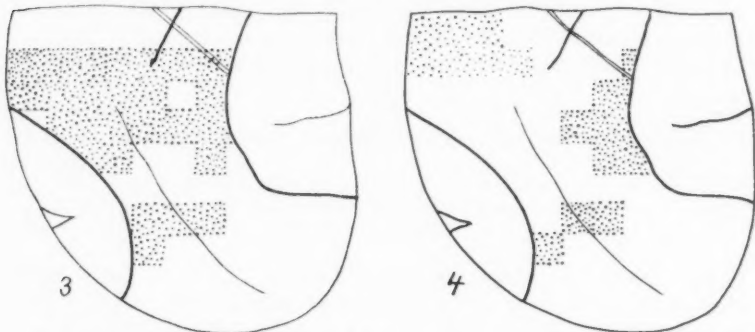


Fig. 3. Areas giving movement of different segments of the arm in different tests. $\times 2$.

Fig. 4. Areas giving movements of different bodily segments in different tests. $\times 2$.

c. There is apparently no functional relation between the movements elicited from the same point at different times. Point d7, for example, gave adduction to the upper arm, rotation of the same segment, shrugging of the shoulder and turning of the eyes with pricking up of the ear in different tests. There is no reason to suppose that such diverse movements represent either parts of a coordinated pattern or of antergic patterns.

3. At different times identical or nearly identical movements may be elicited only from quite widely separated areas. It is difficult to describe movements in such terms that absolute identity can be determined in successive trials. My general impression is that exactly the same pattern of movement is rarely if ever elicited in tests on different days. But movements of the same segment in the same general direction are

recognizable and these were frequently elicited from quite distinct areas at different times. Figures 5 to 12 inclusive show for typical movements areas from which the same movements were elicited in different trials. In all these figures the same symbols for the test are used. Test I, \equiv ; test II, $|||$; test III, $\backslash \backslash \backslash \backslash$; test IV, $////$.

The area for closure of the hand (fig. 5) remained fairly constant in all the tests. Separate adduction of the thumb, obtained in two tests, was, on the contrary, elicited only from widely separated points (*g6* and *e3*, fig. 6). Dorsal flexion of the wrist (fig. 7), flexion of the elbow (fig. 8) and rotation of the forearm (fig. 9), and rotation of the upper arm (fig. 10) all showed wide variations in different trials. Abduction (fig. 11) and adduction (fig. 12) of the arm showed a greater tendency to restriction to one part of the field.

In some of these cases there is overlapping of points in two or more trials but, except for the flexion of the fingers, the distinct areas greatly exceed in extent the overlapping ones. In some cases, as those of rotation of the forearm, abduction and adduction of the upper arm, it might be argued that only one stimuable area is involved and that differences in facilitation or deviation account for the different limits noted. The wide divergence of the areas in figures 6, 7 and 10 make such a general interpretation impossible, however.

4. *There is a tendency toward stability in the grouping of excitable points for the same body or limb segment in the same general area, although the details of movement do not show consistent localization.* Movements involving the leg were never obtained except from the median portion of the field, movements of the face only from the lateral portion. It thus seems possible to distinguish leg, arm and face areas, although with shifting and unstable boundaries. Within the arm area movements of the upper segments tend to be restricted to the median portion of the field, movements of the wrist and hand to the posterior edge of the gyrus, but the amount of variation obtained makes it questionable whether the uniformities encountered are not largely a matter of chance.

THE EFFECTS OF FATIGUE. The attempt to induce fatigue seemed to bring the animal to the end of his ability to make voluntary movements. He had been unusually wild from the first and always struggled violently to avoid handling. After half an hour of continued running he ceased to make any effort to escape and could not be induced to make further attempts at flight by any stimuli that I could apply. It is difficult to determine how much of his inaction was due to actual fatigue and how much to the passivity which the wildest rhesus frequently shows when

finally cornered or held. His last movements, however, were weak and lagging and suggested extreme fatigue. Some recurrence of activity appeared as he was being placed on the operating table, but such a possibility of a final spurt seems to characterize any degree of fatigue,

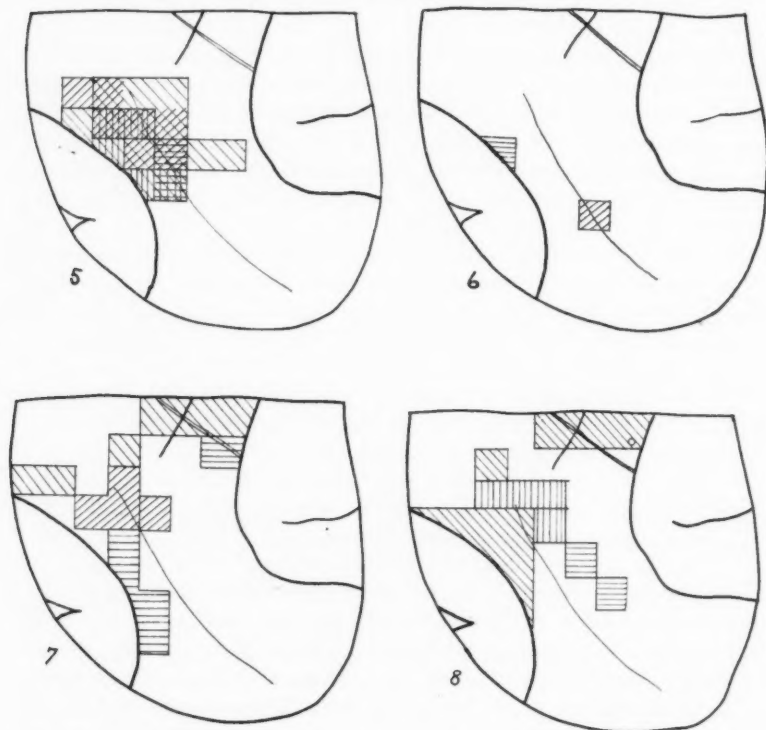


Fig. 5. Areas which gave flexion of fingers in different tests. For significance of cross-hatching see text. $\times 2$.

Fig. 6. Areas giving adduction of the thumb. $\times 2$.

Fig. 7. Areas giving dorsal flexion of wrist. $\times 2$.

Fig. 8. Areas giving flexion of elbow. $\times 2$.

if the incentive can be sufficiently increased. The evidence indicates a rather extreme degree of fatigue, although it can not be characterized as absolute.

The movements elicited from the precentral gyrus in this condition give no evidence that the fatigue had any influence upon the activity

of this area, but rather indicate that the functioning of the area is scarcely affected in fatigue of voluntary movement. After fatigue, 11 diverse movements were elicited by stimulating the exposed area. The average for the three preceding tests is 11.3 distinct movements.

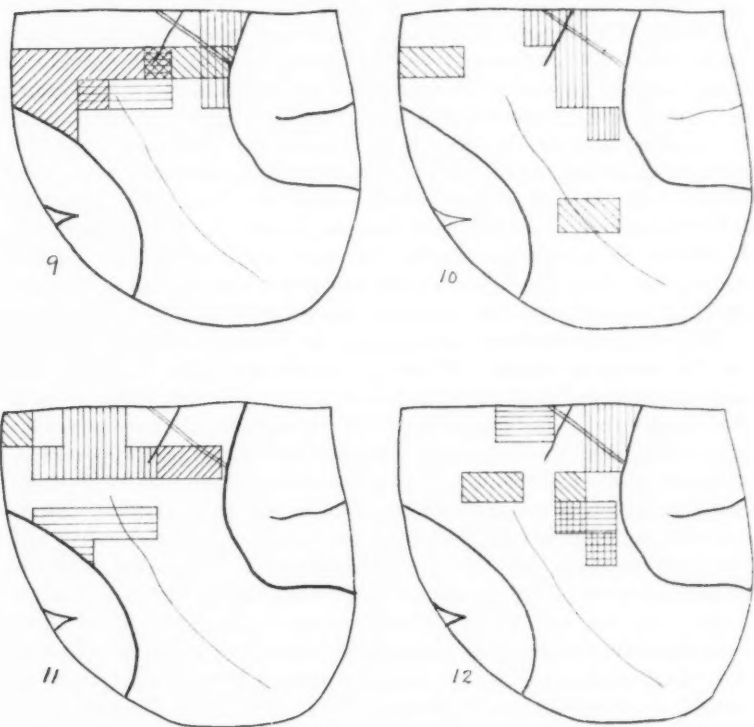


Fig. 9. Areas giving inward rotation of forearm. $\times 2$.

Fig. 10. Areas giving inward rotation of upper arm. $\times 2$.

Fig. 11. Areas giving abduction of upper arm. $\times 2$.

Fig. 12. Areas giving adduction of upper arm. $\times 2$.

The elevation of threshold, although apparently consistent, was not greater than the difference which is frequently found in the same test between adjacent areas.

The last movements elicited in fatiguing were turning the head and eyes to watch the experimenter. The appearance of these movements for the first time in this test might be ascribed to a persistence of a

"set" to this type of response, but similar movements are usually dominant in stimulation experiments just before the animal is anesthetized, so that the assumption of a special relationship in this case does not seem justified.

Even allowing for defects of technique, the experiment points to the conclusion that the incidence of fatigue of voluntary movement is at some point other than at the so-called motor cortex or lower motor neurones. This is in line with the results of many investigations which indicate that the motor end of the reflex arc is relatively unfatigable.

POSSIBLE CAUSES OF TEMPORAL VARIATION. The tests reported show a variation in the functions of the same precentral area from time to time, as great as the diversities found by Franz in the brains of different animals. Is this due to some defect in the experimental technique or to a true variation in functional localization? If the latter, what is the significance of the variation for theories of the organization of the cortico-spinal conduction systems?

The particular methods employed do not seem adequate to account for the variations found. Errors of measurement may have resulted in displacement of points from test to test, but while this might account for variation of adjacent points, it will not explain results such as are shown in figures 6 to 12. Differences in depth of narcosis appeared in different tests, but within a single test approximately the same localization may be demonstrated from the deepest narcosis to the point where avoiding reactions begin to appear and no such wide range was involved in these experiments. Threshold of excitability may be influenced by depth of narcosis, but not localization within any single test.

Irritation of the cortex through successive operations and stimulations may likewise be expected to produce changes in excitability, but to ascribe the variation in function to this is to admit a multiplicity of functional representation which practically abandons the conception of strict and limited localization.

Finally, deviation of response as a result of the cortical stimulation might be urged as the cause of the apparent variation. But the sequence of stimulation was in every case the same during the systematic exploration, and consequently should have produced similar deviating effects. Further, check stimulations were given after the systematic exploration, usually with an intervening rest period. The conception of deviation involves the spontaneous recovery from the effects of deviating influences. Very few changes in function following periods of

rest were noted, and these were not of a character to justify the assumption that they represented a reversion of points to some primary norm of reaction.

It seems clear then that temporal variation must be considered as characteristic of the function of the cortex itself and not as due to the particular technique of this experiment. The experiment is inadequate to test the absolute range of variation either in the localization of specific movements or in the movements which may be elicited from a single point. The areas for different bodily segments remained fairly distinct throughout the tests. This indicates some morphological basis for the differentiation of function in the larger segmental divisions of the gyrus.

Within the arm segment, however, the same points at different times elicited diverse responses and the same response was elicited from widely separated and shifting areas. Within this area there is no justification for ascribing a specific function to individual points or for holding that, within rather wide limits, one movement is more typical than another. The variations were in some cases so extensive as to suggest that, had a greater number of tests been given, an equipotentiality of function of all parts within the arm segments would have appeared. This distinction between segmental areas and the lack of differentiation within the segmental area is in accord with the findings in the paralyses. Monoplegias of cortical origin are fairly common, but cortical paralyses of lesser extent are rare, and in experimental work recovery from them is exceedingly rapid.

Such results can be harmonized with the view which implies a point for point correspondence between the Betz cells and specific patterns of ventral horn cells only by assuming that scattered cells in all parts of a segmental area have duplicated anatomical relationships with spinal mechanisms and that the variations found are due to variations in the threshold of these equivalent cells. The continuity of the pyramidal fibers to different levels of the cords seems to favor this view as far as the distinction between segmental areas and the variations in demarcation between these areas is concerned. But within the segmental area it is equally possible that the character of the response obtained at any given time is determined by the total neural equilibrium, in the sense of Herriek (4, p. 327 ff.). This view would abandon the conception of strict anatomical localization and consider every reaction the algebraic sum of the activities of numberless cells. The present evidence does not permit of a choice between these hypotheses, but does seem

to justify the conclusion that within a segmental area the responses obtained on electrical stimulations are due to the temporary physiological condition and do not give evidence for a corresponding structural differentiation.

SUMMARY

A portion of the precentral gyrus of a rhesus monkey was mapped by electrical stimulation in four tests extending over a period of 18 days.

1. In each test, lasting about half an hour, the reactions were almost constant, subject to slight deviations resulting from the order of stimulation.

2. In the different tests the general fields from which movements of the face, arm and leg segments were elicited tended to remain constant, although the borders of the fields were inconstant.

3. Within the arm area stimulation of the same point in different tests resulted in widely different movements and at different times the same movement was obtained from widely separated and shifting areas.

4. The results suggest that within the segmental areas the various parts of the cortex may be equipotential for the production of all the movements of that area, and that the particular movements elicited in any test depend upon the temporary physiological organization of the area rather than upon any point-for-point correspondence between pyramidal and spinal cells.

5. Fatigue of voluntary movements produced no significant effect upon the excitability of the precentral gyrus or upon the character of the movements elicited from it.

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CONDITIONED REFLEXES AND PATHWAYS IN THE SPINAL CORD

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Received for publication May 25, 1923

The "conditioned reflex," first employed as an experimental method on dogs in Pavlov's laboratory, has been found useful in investigating the functions of the higher centers of the nervous system. It has been found applicable not only to dogs and other mammals including man, but to birds and even invertebrates such as snails, etc. One use to which it has been put is the location of association centers, e.g., that for the discrimination of sounds of different pitch, or of tracts e.g., that for the posture sense. The animal is taught to perform some act at a given stimulus which would not normally be related to that act, for example, the taking of food at a given sound and rejecting it at another. When the association is perfectly formed, a known region of the nervous system is injured or destroyed, and if the conditioned reflex persists, it is concluded that the pathway for the stimulus and its center have not been interfered with. In this manner May and Larsen (1) found that the fibers mediating the posture sense impulses of the dog decussate in part within the cord, and that some of them travel back and forth across the cord at different levels.

It was suggested to us by Dr. J. J. R. Macleod that this method might be employed to investigate in the dog the course of the fibers conveying sensory impulses from the skin, a study which from its subjective character is difficult to carry on in the lower animals. In man it is generally considered that the main tracts cross to the other side of the cord almost immediately after entering it, but that tactile and pressure impulses may ascend in the dorsal funiculus for varying distances and then cross over in the cord gradually, continuing their course in the antero-lateral spino-culmic tracts.

Method. The method followed in our experiments was that described by Watson (2). Dogs only were used as subjects. Since the presence of the experimenter affects the establishment of the conditioned reflex by

bringing variable sets of conditions into play, the dog was tied loosely into a wooden frame in a comfortable position and placed in a room by itself. The experimenter could observe the animal through a small window without being seen or heard by it; electric connections were arranged so that by throwing in various switches the experimenter could cause a bell or a buzzer to sound, he could stimulate the foot of the dog either simultaneously with the sounding of the buzzer, or separately, by means of an electric shock from an inductorium, and also start and stop a kymograph for making records. Attached to the dog's foot was a spring connected with a lever which recorded the least movement of the foot. About the dog's chest was a pneumograph to record respiratory movements. Signal magnets were connected with bell, buzzer and inductorium, and the time was indicated in seconds for a slow record, and in hundredths of a second for fast records.

In the first set of experiments a conditioned reflex to pain was established. The dogs were trained to lift the left hind leg at the sound of the buzzer by sending in an electric shock simultaneously with the sounding of the buzzer. The two stimuli were presented together five times in succession at intervals of ten seconds. The dog was then tested to see if there was response to the buzzer alone. Lessons were given twice daily and lasted 20 to 30 minutes. Training was considered adequate and the conditioned reflex established when the dog lifted its leg to the buzzer alone at the beginning of a lesson. It was found that when this occurred the dog would continue to respond to the sound of the buzzer for ten or twelve times without need for reinforcement by means of the painful stimulus. After the conditioned reflex was thoroughly established the cord was hemisected on the right side at the level of the first lumbar vertebra. This should interfere with the sensory paths on the left side, but leave intact on this, the trained side, the motor fibers coming from the cortex of the brain by way of the crossed pyramidal tracts. When the dog recovered from the shock of operating, trials were made to see if the conditioned reflex persisted. Records were preserved for histological examination. The operations were performed by Dr. N. B. Taylor and the histological preparations made by Dr. E. Horne Craigie.

Eight dogs were trained successfully, but one proved to be so easily disturbed that it could not be depended upon to give the reflex, hence it was not operated upon. Two died too soon after the operation to be tested. Our results are therefore based on the records of five dogs, two of which were submitted to a second hemisection of the cord.

Results. At the first lesson the stimulation of the foot by a faradic shock, though not at all severe, caused general motor activity and a marked irregularity in breathing (fig. 1). Later on in the course of training the activity became confined to the foot stimulated, and the breathing only momentarily disturbed. The conditioned reflex appeared in from 3 to 6 lessons, three of the dogs giving it during the third lesson. The reflex was carried over to the next day after 6 to 11 lessons (fig. 2).



Fig. 1

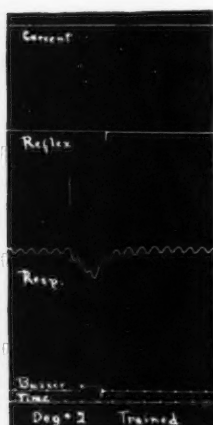


Fig. 2

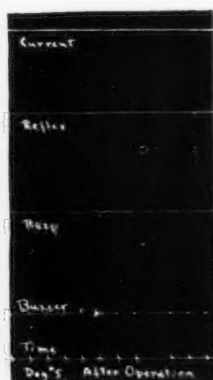


Fig. 3

Fig. 1. First record showing repeated movements of leg and changes in respiration after a single painful stimulus.

Fig. 2. Same dog after training. Reflex shown without painful stimulus.

Fig. 3. Conditioned reflex to pain not given after operation though change in respiration is marked.

Two of the dogs (nos. 2 and 5) were completely paralyzed in both hind legs for over a week after the operation, voluntary movements being quite absent. The conditioned reflex had disappeared, though in one dog change in respiration occurred at the sound of the buzzer, indicating that there was still association of the sound of the buzzer and the painful stimulus. At the end of a week a feeble flexion reflex could be obtained by stimulation of either the right or left foot, showing that the pathway for a reflex involving only the spinal segments

below the lesion was still intact. An attempt to reestablish the conditioned reflex when the dogs were able to use their left hind legs in walking proved unsuccessful, nor was it possible to establish a conditioned reflex to pain in the right hind leg which remained paralyzed. A section of the cord taken just above the level of the lesion showed that hemisection had been complete in these two dogs. In one the degeneration was confined to the right side strictly, in the other there was slight degeneration near the midline in the left dorsal column.

The latent periods for response were measured in dog 6 just before being operated upon. Whenever the conditioned reflex had to be reinforced by the painful stimulus the latent period was fairly constant, and averaged 170σ . But for the conditioned reflex alone the times were much more variable ranging from less than zero to 275σ . Since the signals were thrown in at intervals of 10 seconds merely by watching the second hand of a watch, there must have been variations of the intervals by considerable fractions of a second. When the dog was responding well with the conditioned reflex, i.e., giving 10 to 12 responses without need for reinforcement, it would often anticipate the sound of the buzzer by a fraction of a second. On an average, therefore, the conditioned reflex had a smaller latent period than the true reflex to a painful stimulus.

This dog was able to use its left hind leg 5 days after the operation, the right being completely paralyzed. A slight flexion reflex could be obtained from the left by use of a very strong shock from the inductorium, and had a latent period of 180σ . The conditioned reflex was absent though the respirations showed change at the sound of the buzzer (fig. 3). Three weeks after the operation a heated rod applied to the left hind foot caused no flexion reflex nor was there any change in respiration. Pain was evidently absent from this foot. The same treatment applied to the right leg, which was still completely paralyzed, caused a vigorous flexion reflex, a yelp, and rapid breathing. Training of the left leg was begun again and after 11 lessons the conditioned reflex was reestablished.

The latent periods for the flexion reflex to faradic stimulation in dog 7 was 150σ , quite similar to dog 5, and again the response to the conditioned reflex was quicker, 20σ . In several cases this dog also anticipated the sound of the buzzer by a fraction of a second. Dog 7 was able to be tested the second day after the operation. The right hind leg was completely paralyzed, but the left could bear the weight of the body. There was no movement of the leg to the sound of the

buzzer after operation, but change in respiration was marked. Only a feeble flexion reflex could be obtained in the left hind leg by strong stimulation, but the dog was quite able to move this leg voluntarily. Retraining was effective in 8 lessons, though to establish the reflex so that 5 responses in succession were given took 20 lessons, three times the original time for learning. The dog was then taught to respond with the right leg, and learned in the very first lesson. Lessons were

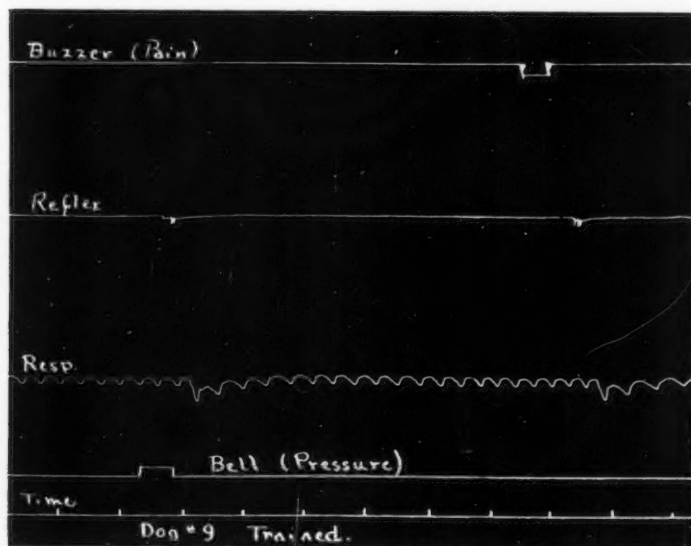


Fig. 4. Dog responds equally well to buzzer and bell without application of pain or pressure stimuli.

discontinued with the right leg, but continued with the left leg. The latent period decreased in a month from 330σ to 70σ for the flexion reflex with stimulation of the foot. The reestablished conditioned reflex began with a latent period of 580σ and at the end of the month was never less than 295σ , the average being 365σ .

This dog was also taught to give a conditioned reflex to pressure with the left hind leg. The stimulus was applied by means of a slight pull on a cord attached to the dog's leg, and at the same time a bell was sounded. At the beginning of this part of the training the dog

responded to the sound of the buzzer, but the bell had no effect whatsoever. The conditioned reflex to pressure was thoroughly established by the 13th lesson and the dog responded equally well to bell and buzzer. The dog was again operated upon, and the cord hemisected on the right side at the level of the 8th thoracic vertebra. Recovery after this operation was not very rapid. There appeared to be slight movement to the sound of the bell, but the result was not perfectly

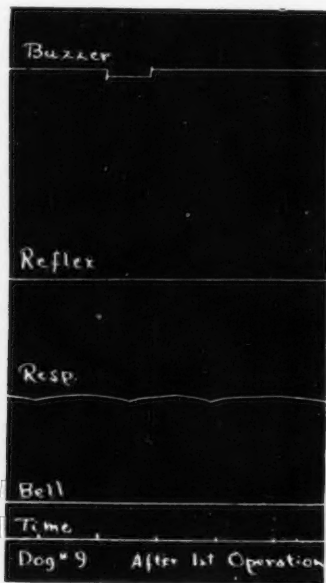


Fig. 5

Fig. 5. No response to substituted pain stimulus after operation. A shorter breath and slight irregularity shown.

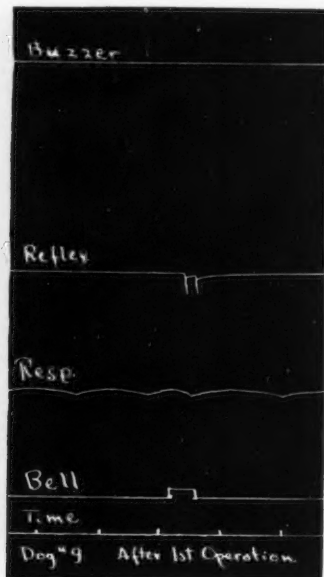


Fig. 6

Fig. 6. Response to substituted pressure stimulus after operation.

conclusive. There was no indication of movement to the sound of the buzzer. The right leg was paralyzed though able to respond with the flexion reflex to pricking and the application of a heated rod. The left leg could be moved voluntarily though it did not respond to pricking or burning.

Dog 9 was also trained to lift the left hind leg at the sound of both bell and buzzer. The bell was used as the substituted stimulus for

pressure and the buzzer for pain. The training with the pressure stimulus was begun first, since it was found that it took a longer time to establish this reflex in the other dog than the conditioned reflex to pain. The reflex first appeared at the third lesson and was carried over at the sixth. When this reflex was thoroughly established the buzzer was tried and found to have no effect. The dog was, therefore, able to distinguish between buzzer and bell. At first the reflex to the bell only was carried over from one lesson to the next; in other words, the process of establishing one reflex was quite distinct from the other. By the eleventh lesson the dog responded equally well to bell and buzzer (fig. 4). The response to the pressure stimulus was greatly delayed, the latent period being 1 to 2 seconds, for the painful stimulus it was 360σ , and for the conditioned reflex quite variable, though considerably less on an average than for the straight flexion reflex. This dog was able to be submitted to the test on the day following the operation. There was a

decided response to the bell, but not to the buzzer (figs. 5, 6). Tests were continued with the conditioned reflex to pressure for 3 weeks. At the end of this time the wound was quite healed. The cord was again hemisected on the right side six vertebrae nearer the head. The dog responded to the bell on the following day (fig. 7).

Discussion. In these experiments it was found that hemisection of the cord at different levels abolished the conditioned reflex to pain but not that to pressure. In agreement with the usual findings after hemisection of the cord there was absence of pain and temperature sense on the side opposite the lesion, but the voluntary motor control on this side was still present. This is quite different from the results

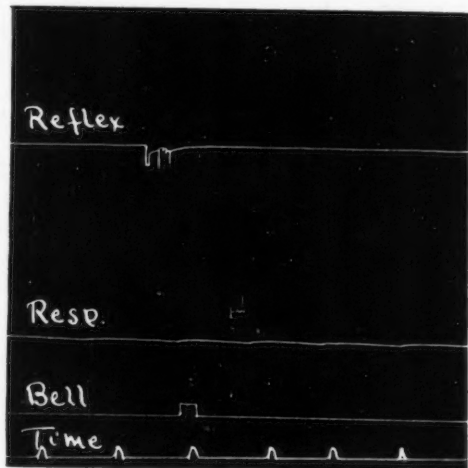


Fig. 7. Response to substituted pressure stimulus after second operation. Note that the change in breathing is hardly more than in figure 5.

of May and Larsen who state that loss of pain sense and also complete motor paralysis occurred on the same side as the lesion after hemisection of the cord, and moreover their dogs gave prompt and decided responses with the completely paralyzed leg to posture tests in which the dogs has been taught to extend the leg rigidly backwards.

In attempting to explain our results on the usual assumption that association takes place in the cerebral cortex, it is difficult to see why the tract for pain in the cord should have to be intact in order for the conditioned reflex to be given, since response occurs apparently without an impulse travelling over this tract, or at least without stimulation to set up an impulse in this nerve. The general conception of a conditioned reflex is that one stimulus is substituted for another, central connections being made so that the motor pathway is the same for both reflexes. If a response is given to the substituted stimulus, one would think that interference with the pathway in the cord for the original stimulus could make no difference whatever, for it is evident that the entire pathway from the point of reception of the substituted stimulus over the connecting fibers in the brain to the motor nerves must be functioning properly, since the leg moves at the given sound.

Another difficulty presents itself when one attempts to explain how the dog is able to be retrained to give the conditioned reflex to the same stimulus after it had once been abolished by cutting the cord, and when the sense of pain is gone in that leg. It may be that in this case the pain fibers which were still able to act on the ventral horn cells of the same segment—shown by the fact that the flexion reflex could be given—established connection with the cortex through intermediary cells on the other side of the cord. An indication that the pathway concerned in this reflex was more devious than before cutting the cord is given by the increase in the latent period for this reestablished conditioned reflex. This again presents the difficulty noted above. There is no apparent reason why the pathway for the conditioned reflex, if it involves only the course of the nervous impulse from the reception of the substituted stimulus through the cerebral cortex to the motor nerves, should be so evidently different before and after the first operation, and then should again be abolished by cutting the cord higher up. It appears that the whole mechanism must be complete for the conditioned reflex to take place, i.e., the pathways for both sets of stimuli must be intact. This would be the case for the conditioned reflex to pressure after both low and high hemisection of the cord if

the fibers did not cross until the cervical region, whereas if the pain fibers cross at once section of the cord at any level above the segment at which they enter would abolish the reflex.

SUMMARY OF RESULTS

1. A conditioned reflex to pain involving the left hind leg in dogs was abolished by hemisection of the cord at the level of the first lumbar vertebra on the side opposite to the trained leg.

2. The reflex could be reestablished after a comparatively long period of training, although the sensation of pain was apparently absent. The latent period of the reestablished reflex was much longer than that of the original conditioned reflex.

3. The reestablished conditioned reflex was abolished by a second hemisection of the cord on the opposite side several segments nearer the brain.

4. A conditioned reflex to pressure involving the same motor mechanism as that to one for pain was retained after both low and high hemisection of the cord on the opposite side.

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INFLUENCE OF TEMPERATURE CHANGES ON THE SECRETION OF EPINEPHRIN¹

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Received for publication May 12, 1923

It has been shown by Hartman, McCordock and Loder (1) that stimulation by cold causes a marked increase in the output of epinephrin. This appeared to be due either to a lowering of body temperature or to peripheral stimulation or both. We have attempted to throw more light on this question.

The completely denervated pupil as in the previous study (1) has been used to indicate an increase in the output of epinephrin. The pupil was measured by a caliper square graduated to hundredths of an inch. The *transverse* diameter was always measured. The animal was usually studied within two or three days after removal of the ciliary ganglion while the iris was most sensitive. The superior cervical ganglion had been removed several days before the ciliary operation. Cats were used exclusively.

Excitement does not appear to be an important factor in the increase of epinephrin from cold in most instances. We kept many of our animals and worked with them sufficiently so that they were accustomed to being held in the most awkward positions. They usually did not object to being immersed in water, either warm or cold. They sometimes struggled after becoming chilled but whether they struggled or not the epinephrin increase was shown. If firmly held they struggled comparatively little. Occasionally they struggled as much in water at body temperature as in colder water, yet there would be no epinephrin increase. Moreover in experiments where the skin was merely soaked with water, the animal then being allowed to stand in the air current from an electric fan, an increase in epinephrin was shown without the appearance of excitement.

The effect of cold on the epinephrin output therefore seems to be largely independent of excitement.

¹ This study was aided by a grant from the Elizabeth Thompson Science Fund.

We have attempted to find out whether the epinephrin increase is due to cooling of the heat center or to peripheral stimulation. Peripheral stimulation by cold without reduction in the temperature of the heat center has been produced by a block of ice and a hot water bag, the latter being used to compensate for the loss in general body temperature caused by the ice.

The following experiment gives an idea of the behavior of the adrenals in response to temperature change. A cat (239) with one iris completely denervated (the ciliary ganglion had been removed seventeen hours before) was stimulated first by a block of ice in contact with about 70 sq. cm. of skin. The fur was soaked to facilitate conduction. A hot water bag ($60^{\circ}\text{C}.$) was placed near the animal on the opposite side in order to maintain a constant internal temperature and thus rule out direct stimulation of the heat center by cooled blood. The rectal temperature was watched constantly. The position of the hot water bag was changed so that the rectal temperature did not vary more than $0.1^{\circ}\text{C}.$ The animal was held on its back by an assistant. Perfect docility was shown by this cat. No change in the pupil occurred for six minutes, the diameter being 3.81 mm. at the beginning. At the end of ten minutes the pupil had increased to 4.32 mm., where it remained for eight minutes. By the end of twenty minutes the pupil had increased to 4.83 mm. The ice was then removed. The cat was next slowly immersed in water at $46^{\circ}\text{C}.$ In five minutes the respirations had increased to 114 per minute (normal 36). The rectal temperature, however, remained at $39.4^{\circ}\text{C}.$ In seven minutes the respirations had become 210 per minute while the rectal temperature had increased to $39.7^{\circ}\text{C}.$ At the end of ten minutes the cat was removed from the bath. During this time the diameter of the denervated pupil had not changed (remaining at 4.83 mm.) while the rectal temperature had not gone as far as $39.8^{\circ}\text{C}.$

The cat after a thorough soaking was placed in a current of air made by a sixteen inch electric fan. In eight minutes the rectal temperature had dropped to $38.3^{\circ}\text{C}.$, but the pupil had not changed. In eleven minutes the cat began to shiver slightly. In sixteen minutes the pupil had increased to 5.33 mm., while the rectal temperature was $38.2^{\circ}\text{C}.$ At the end of twenty-four minutes the rectal temperature had dropped to $37.4^{\circ}\text{C}.$, while the pupil had dilated to 7.62 mm. The cat was soaked again at this stage. After thirty-two minutes (eight minutes after the second soaking) the pupil had increased to 8.64 mm., with the rectal temperature at $36.8^{\circ}\text{C}.$ After thirty-nine minutes the diameter of the

pupil had been reduced to 7.11 mm. Two minutes later the cat was soaked. Five minutes after the third soaking the diameter of the pupil had again become 8.64 mm., with the rectal temperature at 36.2°C. But twelve minutes after the third soaking the diameter of the pupil had been reduced to 7.11 mm., and the rectal temperature to 35.3°C. The pupil then dilated a small amount (to 7.62 mm.) during the following fourteen minutes. At the end of this period (twenty-six minutes after dipping) the temperature had fallen to 34.8°C. Another soaking (twenty-eight minutes after the preceding) caused an increase of the pupil to a diameter of 8.89 mm., and a rectal temperature of 34.2°C. Dipping again (fifteen minutes after the preceding) and evaporation caused the diameter of the denervated pupil to increase to 11.16 mm. in five minutes. Repetition of the dipping (seven minutes after the preceding) increased the pupil to 11.41 mm. in five minutes (rectal temperature 33.0°C.) The fan was stopped and the cat rubbed as dry as possible. Five minutes after stopping the fan the diameter of the pupil had been reduced to 8.37 mm. while the temperature had dropped to 32.6°C. The cat was next placed near an electric heater. Seven minutes afterward the diameter of the pupil had become 6.34 mm., while the rectal temperature was 32.5°C. After eighty-seven minutes' exposure to this heat the rectal temperature had returned to normal (39.4°C.) and the diameter of the denervated pupil had become 4.06 mm. The heater was then removed. Sixty-five minutes after removal of the heater the temperature had dropped to 38.7°C. and the pupil had constricted to 3.30 mm.

This experiment seems to show that stimulation by cold of a small area of the skin can cause a small increase in the epinephrin output. That this may occur independently of a reduction of temperature in the heat center is demonstrated by artificial compensation for the heat loss. That peripheral stimulation is an important factor in the epinephrin increase from stimulation by cold is further indicated by the decided increases in epinephrin output (pupillary dilatation) following each dipping in water without very decided increases in the rate of fall in body temperature (fig. 1). The much larger areas of skin stimulated when the whole cat was soaked might account for the greater increase in epinephrin rather than the general decrease in body temperature. In fact, the variations in epinephrin output seem to go hand in hand with variations in peripheral stimulation.

On the next day loose ligatures were placed under each adrenal vein after tying the vein from the lumbar muscles in each instance.

Each of these ligatures was passed through separate sterile rubber tubes long enough to reach from the adrenal to a point well outside of the body cavity. The opening in the abdomen (mid-line) was sewed up with the tubes containing ligatures protruding. This arrangement permitted the occlusion of one or both adrenal veins at will without interfering with other organs. The tubes and ligatures were protected by sterile cotton and bandages until ready for use. Three and one-half hours after the operation the following experiment was tried, the animal being no longer under the influence of the ether.

Both adrenal veins were occluded as much as possible by traction on the ligatures. This caused the diameter of the pupil to decrease from 5.08 mm. to 1.78 mm. in five minutes. The epinephrin shut off from

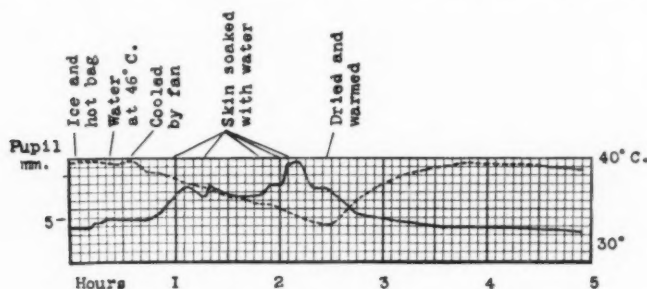


Fig. 1. Response of completely denervated pupil when cat 239 was subjected to variations in temperature. Broken line, rectal temperature. Solid line, transverse diameter of pupil. Note that the abrupt increases in the pupil follow fresh soaking of the skin.

the circulation by this occlusion was probably being secreted as a result of stimulation from the injured tissues (see operative procedure Hartman, McCordock and Loder). At the end of fourteen minutes, no further change being shown, the animal's back was soaked with water. The electric fan was used to increase the rate of evaporation. In three minutes from the time of dipping, the pupil had reached the diameter of 4.06 mm. In eleven minutes the diameter had become 4.83 mm. At this stage the ligatures were tightened anew with the result that the diameter decreased to 2.29 mm. in seventeen minutes. Dilatation again occurred until a diameter of 5.33 mm. had been reached. A third attempt to tighten the ligatures decreased the diameter of the pupil to 4.57 mm. in two minutes. The ligatures were then released.

The dilatation increased within a few seconds and reached the maximum of 7.35 mm. in two minutes. It then gradually decreased to 6.60 mm., where it remained for seven minutes.

The fan was stopped. The cat was dried, wrapped up and placed near a heater. In twelve minutes the diameter of the pupil had become 5.08 mm. It remained at this size as long as it was watched, two and one-third hours after placing near the heater. This experiment would seem to prove that the adrenals are responsible for the increased dilatation of the denervated pupil by cold. It is not surprising that the adrenals could not be completely occluded for the whole time desired because movements of the animal would tend to release the tension of the ligatures. The rate of temperature decrease was as great as in the preceding experiment. (The temperature fell as low as 32.7°C.)

We attempted to distinguish peripheral effects from direct effects on the heat center by another experiment in which the rectal temperature was kept nearly constant through compensation for the heat loss. Cat 226 was partly immersed in water at 17.6°C. Heat from an electric heater was directed upon the part of the animal out of the water, care being taken to shield the rectal region and head in order to avoid local effects upon the thermometer and iris respectively. The rectal temperature was 38.0°C. at the start and the same at the finish twelve minutes later. The maximum temperature reached was 38.2°C., while the minimum was 38.0°C. The diameter of the pupil was 8.64 mm. at the start and 10.66 mm. at the finish (fig. 2). Four hours later, as a control, the cat was immersed in water at 38.4°C. for ten minutes. The animal struggled as much if not more than in the preceding test. The rectal temperature increased from 38.5°C. to 39.0°C., while the diameter of the denervated pupil increased slightly, from 8.89 mm. to 9.14 mm. After a thorough soaking the animal was removed to an open window. The diameter of the pupil gradually increased until it had reached 11.16 mm. in forty-five minutes. In the meantime the rectal temperature had dropped to 35.7°C. The maximum increase in the diameter of the pupil was little greater in this test than in the preceding although the rectal temperature fell 3.2°C. Moreover it required a much longer time to reach this maximum. This was probably due to the slower cooling. The larger area of skin stimulated did not more than compensate for the weaker stimulus used.

Finally we tried the effect of intense stimulation of a small area of skin, compensating for the loss in body temperature by means of artificial heat. In this test a warm water bag was applied to one side

and a block of ice to the other. The rectal temperature fluctuated between $38.0^{\circ}\text{C}.$ and $37.6^{\circ}\text{C}.$ At the close of the experiment the temperature was $37.9^{\circ}\text{C}.$ while the diameter of the pupil had increased from 8.64 mm. to 10.16 mm. This increase is not so great as in the first test when approximately one-half of the animal was immersed in cold water, although the fluctuation of the rectal temperature was slightly greater. This is probably explained by the smaller area of skin stimulated. Experiments with this cat certainly indicate that cold has a peripheral action in causing an increase in epinephrin.

We tried the effect of immersion in water at different temperatures on two more cats.

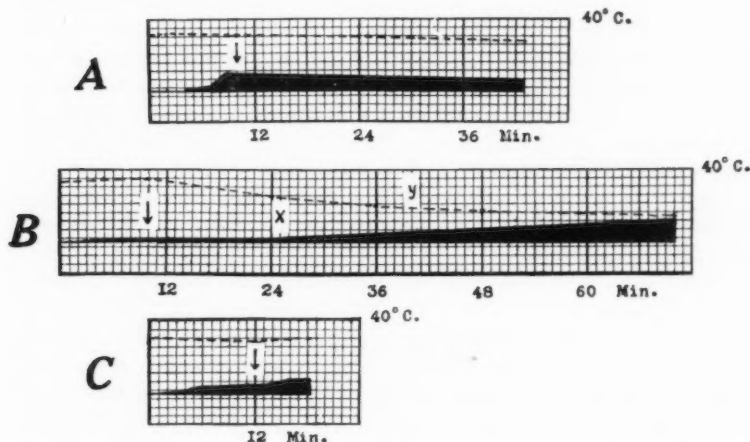


Fig. 2. Increase in the diameter of the completely denervated pupil due to stimulation by cold (cat 226).

Pupil increase in solid black; 1 division = 1 mm. dilatation.

Rectal temperature, broken line; 1 division = $1^{\circ}\text{C}.$

A: Marked cooling of a large area of skin produced by partial immersion in water at $17.6^{\circ}\text{C}.$ Lowering of general body temperature prevented by warming with an electric heater, the part of the animal not immersed. Immersion and heating began at the very first of the graph. Removed from bath and away from heater at \downarrow and exposed to room temperature of $25^{\circ}\text{C}.$, without drying.

B: Moderate cooling of a large area of skin produced by exposure in air at $25^{\circ}\text{C}.$, (\downarrow to X) after immersion in water at 38° to $37^{\circ}\text{C}.$ for ten minutes. From X to the end of the experiment the cat was placed by an open window. At Y the skin was again soaked.

C: Intense cooling of a small area of skin (about 50 sq. cm.) produced by a block of ice. Lowering of general body temperature prevented by warm water bag applied to opposite side of animal. Ice removed at \downarrow .

In the first (224), water at 45°C. caused an increase in the completely denervated pupil from 4.57 mm. to 5.33 mm. after six minutes' immersion. The rectal temperature increased from 39.0° to 40.1°C. The animal began to pant. This small increase in the pupil could be accounted for by an increase in the temperature of the iris (see Hartman, McCordock and Loder).

Transfer of the animal to a bath at 39.7°C. caused no change in the pupil in ten minutes. The rectal temperature however returned to 39.0°C.

The wet cat was then exposed to air at 19.7°C. This caused an increase in the diameter of the denervated pupil to 9.39 mm. in ten minutes, from 5.33 mm. The rectal temperature had decreased to 37.7°C. Six hours after removal from the bath the denervated pupil was still larger than normal (6.09 mm., while the rectal temperature had risen to 38.8°C. This experiment seems to demonstrate that heat (45°C.) does not cause a liberation of epinephrin. It also confirms our previous interpretation that it is the cooling and not the excitement from wetting which causes the release of epinephrin.

Similar results were obtained in the second cat (225). Immersion in a bath at 45.3°C. for eleven minutes caused no increase in the denervated pupil (7.87 mm.)

On the other hand immersion of this cat in a bath at 18.5°C. caused an increase in the diameter of the denervated pupil to 10.41 mm. in fifteen minutes. In three minutes from the beginning of the immersion the denervated pupil began to dilate (becoming 8.12 mm.). Shivering was not detected until seven minutes had elapsed although the pupil had become 8.37 mm. At the end of this immersion period the rectal temperature had become 32.5°C. One hour and fourteen minutes after removal of the animal from the bath the pupil had returned to normal size, while the rectal temperature had risen to 38.2°C. (normal 39.0°C.).

In some instances of cooling, shivering and dilatation of the denervated pupil begin approximately at the same time. The shivering in its early stage is first noticed by those holding the animal. As the shivering increases it can sometimes be seen as well as felt. The dilatation of the pupil usually begins as early or earlier than the shivering. The muscular activity during shivering and the occasional struggling are never sufficient to account for the increase in epinephrin secretion, judging from the amount of work which is necessary to show such an increase in the treadmill (2).

POSSIBLE INFLUENCE OF THE EPINEPHRIN INCREASE ON BLOOD CONCENTRATION. In connection with the influence of cold on the output

of epinephrin a suggestion made by Barbour (5) is worth considering. Barbour (3) found that there is a shifting of water from the blood stream to the tissues when an animal is exposed to cold. The nervous system plays an essential part in this adjustment (4). He suggested that epinephrin might also play a part in driving water from the circulation. He had no proof that epinephrin secretion is increased during stimulation by cold. It was known (6) that massive doses of epinephrin can cause shifting of fluid from the blood stream. Knowing now that the epinephrin output is increased from stimulation by cold, the question is whether the amount is sufficient to drive fluid from the blood. We tried to find the answer by a comparison of the effect of cold on blood concentration before and after epinephrectomy.

Blood was drawn from the marginal ear vein into weighed glass tubes closed at one end. The blood was weighed immediately after collection (about 0.1 to 0.2 gram was collected in each sample). It was then dried for a few days in a desiccator, over sulphuric acid.

Cat 245 (weight 2.63 kgm.) gave the following results when cooled by immersion in water. The animal struggled much throughout the experiment.

TIME	RECTAL TEMPERATURE	PER CENT SOLIDS IN BLOOD
11:05 a.m.	39.7°C.	18.01
11:16 a.m.	Immersed in water at 8°C.	
11:26 a.m.	39.7	19.55
11:36 a.m.	38.9	20.04
11:46 a.m.	38.1	19.80
11:56 a.m.	38.0	20.19
12:06 p.m.	37.0	19.90
12:16 p.m.	36.5	19.92

Both adrenals were removed fifteen days later at 10 a.m. The following test was then made.

TIME	RECTAL TEMPERATURE	PER CENT SOLIDS IN BLOOD
1:19 p.m.	37.8°C.	22.57
1:20 p.m.	Immersed in water at 14°C.	
1:30 p.m.		23.44
1:40 p.m.	34.1	23.43
1:50 p.m.		23.63
2:00 p.m.	30.0	23.55
2:05 p.m.	Shivering	
2:10 p.m.		23.45
2:20 p.m.	28.0	23.90

This animal certainly showed as great an increase in concentration of the blood from cold after double epinephrectomy as before. Cat 244 likewise showed as great an increase in the concentration of the blood in response to cold after double epinephrectomy as before.

The first test gave the following results:

TIME	RECTAL TEMPERATURE	PER CENT SOLIDS IN BLOOD
10:56 a.m.	39.7°C.	15.27
10:57 a.m.	Immersed in water at 18°C.	
11:02 a.m.		15.80
11:07 a.m.	Shivering	17.20
11:16 a.m.	35.6	
11:18 a.m.		17.10
11:24 a.m.	32.3	

Four days later a second test was made. One iris had been completely denervated. The iris was too large, however, to be very sensitive. The results follow.

TIME	RECTAL TEMPERATURE	PER CENT SOLIDS IN BLOOD	PUPIL mm.
11:35 a.m.	39.3°C.	17.0	
11:45 a.m.	Immersed in water at 15°C.		
11:50 a.m.	39.8		10.66
11:52 a.m.	39.45		
11:56 a.m.	39.4	17.1	
12:01 p.m.	39.0		10.66
12:06 p.m.	38.7	17.34	10.66
12:09 p.m.	38.5		10.66 Shivering started
12:16 p.m.	38.1	17.84	10.66
12:19 p.m.	37.8		10.66 Struggles
12:23 p.m.	37.7		10.66
12:26 a.m.	37.2	18.08	10.66
12:31 p.m.	36.9		11.16
12:36 p.m.	36.6	17.98	11.16
12:42 p.m.	36.4		11.16
12:46 p.m.	36.4	17.67	11.16

Two days later both adrenals were removed. Three hours after this the following test was made.

TIME	RECTAL TEMPERATURE	PER CENT SOLIDS IN BLOOD	PUPIL <i>mm.</i>
3:18 p.m.	37.8°C.	17.89	10.16
3:24 p.m.	Immersed in water at 13.5°C.		
3:33 p.m.	Shivering		
3:34 p.m.	37.8	18.1	10.16
3:44 p.m.	37.25	18.83	10.41
3:54 p.m.	36.9	18.73	10.41
4:04 p.m.	36.4	19.2	10.41
4:05 p.m.	Struggling		
4:10 p.m.	Shivering greatly		
4:14 p.m.	36.1	19.4	10.41

Although our experiments are not numerous enough to be conclusive, they furnish no evidence that the adrenals are factors in the concentration of blood in response to cold. It would appear that the amount of epinephrin set free by stimulation from cold is too small to be effective in this respect.

Our experiments seem to show that heat (below 45°C.) does not increase the output of epinephrin, but that cooling of the skin increases this output. The magnitude of this increase appears to depend upon the amount of surface stimulated. Although it is possible that a general fall in body temperature may be a factor, peripheral stimulation appears to be the more important.

The amount of epinephrin released does not appear to be sufficient to materially influence the increased concentration of the blood in response to stimulation by cold.

SUMMARY

By the use of the completely denervated iris as an indicator of increased epinephrin secretion, the following observations have been made.

Immersion of cats in water ranging from 39° to 45°C. does not appear to increase epinephrin discharge. Cooling the skin of an animal by ice, by immersion in cold water or by evaporation of water from the skin causes an increase in the output of epinephrin. Although sometimes accompanied by excitement and struggling, the increased epinephrin discharge can take place without these. Therefore, cold seems to produce an effect of its own and does not do so through the mediation of excitement or muscular activity.

Peripheral stimulation rather than the falling general body temperature seems to be the cause of the increase in epinephrin resulting from cooling. The magnitude of the increased output of epinephrin seems to depend upon the intensity of the stimulus and the extent of the skin area stimulated.

No evidence could be found that the increase in the output of epinephrin is sufficient to play a part in the concentration of the blood resulting from cooling.

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THE PRODUCTION OF EPINEPHRIN BY THE ADRENAL CORTEX¹

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Received for publication May 16, 1923

Epinephrin is the only specific substance definitely known to be produced by the adrenal gland. It is found concentrated in the medulla. It has been assumed to be a product of the medulla. It is quite possible that it is manufactured in the cortex and stored in the medulla or it might be manufactured in both cortex and medulla and stored in the latter. In the present instance we shall consider the possible production of epinephrin by the cortex.

The experiments of Toujan (1), in which he claimed a 30 per cent increase in epinephrin when the cortex was incubated, are unsatisfactory because the test which he used was not specific for epinephrin. Incubation possibly produced other substances which give the iodine test. Voegtlin and Macht (2) obtained a vasoconstrictor substance from the cortex. This was not epinephrin for it was not destroyed by boiling with weak alkali.

The first real evidence that epinephrin is produced in the cortex is found in the work of Cramer (3). He fixed thin slices of the adrenal with osmic acid. The fat and lipoids were removed by turpentine. The epinephrin granules remained. He found fine black granules, similar to the epinephrin granules of the medulla, in the cortex especially in the layers nearest the medulla.

We have been able to obtain positive tests for epinephrin in the cortex by the Folin, Cannon and Denis (4) colorimetric test, by the intestinal inhibition test, by the "sensitized pupil exercise" test and by the "denervated pupil-excitement" test.

Production of epinephrin by the cortex as indicated by the colorimetric test. The presence of epinephrin in the cortex can be easily demonstrated by the Folin, Cannon and Denis colorimetric test. When very

¹ This study was aided by a grant from the Elizabeth Thompson Science Fund.

small amounts of epinephrin are involved as in a fragment of cortex, all of the supernatant liquid should be used after centrifuging. After development of the color from treatment with the phosphotungstic reagent and sodium carbonate, 10 cc. or 25 cc. volumetric flasks were used. The color standard was made up in a similar flask to nearly match the unknown, final comparison being made with a Duboseq colorimeter.

In the first experiment, the adrenals of a cat (A) killed by an overdose of ether were used. The cat had been kept in the cold two days after killing. The left adrenal was carefully split longitudinally the broad way by means of a razor. Some of the cortex was then carefully clipped away with sharp scissors, enough being left to insure an absence of medulla in the cortex removed. The whole adrenal weighed 0.16 gram. The cortex used for separate determination weighed 0.115 gram, and contained 0.067 mgm. of epinephrin. The medulla with adhering cortex weighed 0.045 gram and contained 0.082 mgm. of epinephrin. The right adrenal was not split as it was thought that epinephrin might be lost in this way. The cortex was merely clipped away with sharp scissors, care being taken as before to avoid removal of medullary tissue. The whole gland weighed 0.155 gram. The cortex used for separate determination weighed 0.098 gram and contained 0.067 mgm. of epinephrin. The medulla with adhering cortex weighed 0.057 gram and contained 0.118 mgm. of epinephrin.

From these observations it appears that epinephrin is found in the cortex. It might be argued, however, that this epinephrin might have come from the medulla. In order to rule out the possibility of forcing epinephrin from the medulla to the cortex by manipulation, the adrenal of another cat (B) was carefully removed and frozen with carbon dioxide. While frozen the outer part of the cortex was shaved away with a sharp scalpel on the side opposite the adrenal vein, care being taken to avoid the medulla. The whole adrenal weighed 0.54 gram. The cortical tissue used for separate determination weighed 0.15 gram and contained 0.044 mgm. of epinephrin. The remainder of the gland (0.39 gram) consisting of medulla and cortex contained 0.235 mgm. of epinephrin.

We tried to show more conclusively whether epinephrin is produced in the cortex by destruction of the medulla with an electric cautery.

The adrenals were exposed either through an opening made in the linea alba or through transverse openings beneath the ribs on either side, in the latter case the animal being shifted from one side to the

other to expose each adrenal in turn. The lumbar vein was tied and cut so that the gland could be handled more easily. The cauterizing instrument was made by doubling a piece of nichrome wire sharply on itself and then reducing the diameter of the wire for about 1 cm. in this region by filing. A suitable current sent through this wire made the thin region very hot. The nichrome wire was attached to copper wire at both ends and then fixed to a handle. The cauterizing instrument together with the attached copper wire was always sterilized. The operations were entirely aseptic and the site of the operation was protected by sterile bandages afterward.

Adrenals triangular in shape were usually cauterized through two openings, the hot wire being plunged through the cortex at two of the corners in turn so far as to make sure of destruction of the medulla. Likewise the wire was turned from side to side in broad parts of the adrenal. Elongated adrenals were cauterized through one end, the wire being turned from side to side. By using a very hot wire hemorrhage was usually brief or absent, because the carbonized material formed a plug as the wire was withdrawn.

Later the cauterized adrenals were removed and cut transversely into five approximately equal parts. The second and fourth parts were fixed by formaldehyde and potassium bichromate, sectioned with a freezing microtome and after staining with hematoxylin examined for medullary tissue. The first, third and fifth parts were used for the colorimetric determination of epinephrin.

As might be expected, we failed to destroy all of the medulla in some instances. In order to do this it was necessary to destroy a considerable part of the cortex. Occasionally the destruction was so complete that that none of the cortex survived.

Both adrenals were thoroughly cauterized in the medullary region in cat 179. Death occurred about 14 hours later. The medulla was found to be completely destroyed in the right gland. The cortex contained many vacuoles and lipoids had almost disappeared from the zona glomerulosa. This adrenal weighed 0.183 gram. The portion (0.085 gram) used for epinephrin determination contained 0.014 mgm. of epinephrin. According to this 0.183 gram would contain about 0.03 mgm. of epinephrin. The poor condition of the cortex would account for the very low amount of epinephrin.

The left adrenal (0.185 gram) contained more healthy cortex but unfortunately also contained a fragment of the medulla. The portion used for colorimetric determination (0.1 gram) contained 0.04 mgm. of

epinephrin. According to this the left adrenal contained 0.074 mgm. of epinephrin.

Cat 111 possessed but one adrenal, the other having been removed several months previously. This adrenal was cauterized thoroughly from both ends. The animal died 24 hours later. It was then frozen until the determination was made (20 hours later). All of the medulla and much of the cortex had been destroyed. The portion of the adrenal used for colorimetric determination (0.43 gram) gave 0.027 mgm. of epinephrin. This is not surprising because so much of it was dead tissue.

Both adrenals were thoroughly cauterized in cat 180. The animal died 2 days later. The left adrenal weighed 0.335 gram. The medulla appeared to be absent although the gland was so congested with blood that it was not possible to be absolutely certain. A piece of this gland 0.22 gram contained 0.022 mgm. of epinephrin.

Cat 182 lived 22 days after cautery of both glands. The right adrenal weighed 0.105 gram. Epinephrin to the extent of 0.0133 mgm. was found in 0.084 gram of this gland. There was a much smaller amount of healthy cortex present than in the left gland. No medulla could be found. The left adrenal weighed 0.102 gram. Epinephrin to the extent of 0.03 mgm. was found in 0.085 gram of this gland. A slight amount of medullary tissue was found.

Cat 188 was killed 9 days after cautery of the adrenals. The right adrenal weighed 0.290 gram. Part of the cortex was stripped away from the plug which had been produced by cauterization. The cortex used weighed 0.143 gram and contained 0.031 mgm. of epinephrin. The plug weighed 0.05 gram and gave no blue color whatever when tested for epinephrin. No medulla was found upon microscopic examination of a cross section of this gland.

A portion of the medulla remained in the left adrenal. A piece of this gland, 0.177 gram, gave 0.095 mgm. of epinephrin.

Cat 184 was killed 10 days after cautery of the adrenals. The medulla was completely destroyed in the right adrenal, while a small amount of medulla had escaped destruction in the left adrenal. A piece of the right adrenal, 0.22 gram (whole gland weighed 0.273 gram) contained 0.016 mgm. of epinephrin. Two determinations were made in the case of the left adrenal. The cautery plug together with some good cortex weighed 0.215 gram (whole gland weighed 0.35 gram) and contained 0.027 mgm. of epinephrin. A piece of good cortex alone, 0.085 gram, contained 0.029 mgm. of epinephrin.

Cat 177 died 26 days after cautery of the adrenals. The right adrenal contained a very few medullary cells. The left adrenal contained no medulla. A piece of the right gland, 0.10 gram (total weight, 0.145 gram) contained 0.012 mgm. of epinephrin. The left adrenal appeared to have more healthy cortex. Epinephrin to the extent of 0.04 mgm. was found in 0.113 gram of gland (total weight, 0.155 gram).

The right adrenal of cat 176 was removed 30 days after cautery. A plug of cauterized tissue, making up a large part of the gland, was easily separated from the small layer of healthy cortex. The cauterized part was so extensive that none of the medulla could possibly have escaped destruction. The plug weighed 0.055 gram and gave no color whatever when tested by the Folin, Cannon and Denis method. The cortical shell was so thin and friable that a relatively considerable amount of connective tissue was left adhering to it to keep it together. Cortex and connective tissue weighed 0.037 gram and contained 0.011 mgm. of epinephrin.

Examination of table 1 shows the amount of epinephrin found in the cortex of the different individuals. Usually much more was found when medullary tissue was present. Frequently the medullary tissue present was so small in amount that it scarcely accounted for the greater proportion of epinephrin. It seems rather that the better condition of the cortex accounted for the difference. The amount of epinephrin per gram of cortex was small, ranging from 0.063 to 0.341 mgm., in glands containing no medulla. The amount of epinephrin per gram of healthy cortex was actually greater than this for much dead or inactive tissue was used in the determination. Perhaps the amount of epinephrin obtained from the cortex of uncauterized glands gives a better idea of the epinephrin content of healthy tissue. In any case the amount of epinephrin found in the cortex at any one time is very small.

Production of epinephrin by the cortex as indicated by the intestine test. We have also tested the adrenal cortex for the presence of epinephrin by means of the intestine method. Material to be tested was ground in an agate mortar and extracted with mammalian Ringer's solution. In order to reduce to a minimum the dilution of the extract, very small T-tubes having a capacity of about 1.0 cc. were used. Pieces of intestine from young kittens or rats were used because of their small size. The intestine occupied a considerable portion of the tube so that much less than 1.0 cc. of extract was required. The extract to be tested was always oxygenated and brought to the same temperature as the solution

containing the intestine before being introduced into the tube holding the intestine. Extract of spleen was sometimes used as a control.

An adrenal of a cat recently killed was frozen with carbon dioxide. Some of the cortex was then shaved away with a sharp scalpel, care being taken to remove only the outermost part. The extract of this material caused inhibition of a piece of kitten's intestine contracting in Ringer's solution.

TABLE I

Epinephrin in the cortex of different cats as shown by the colorimetric method

CAT	ADRENAL	TIME IN DAYS BETWEEN CAUTERIZA- TION AND DETER- MINATION OF EPINEPHRIN	AMOUNT OF MEDULLA PRESENT	CONDITION OF CORTX	EPI- NEPHRIN PER GRAM OF CORTX
		<i>days</i>			<i>mgm.</i>
A	Right	Cortex merely cut away 0	None		0.683
A	Left	Cortex merely cut away 0	None		0.582
B		Cortex merely cut away 0			0.293
179	Right	0.6	None	Fair	0.164
179	Left	0.6	Fragment	Better than right	0.40
111		1	None	Very poor	0.063
180		2	None	Poor	0.10
188	Right	9	None	Fair	0.216
188	Left	9	Small portion	Better than right	0.536
184	Right	10	None	Fair	0.073
184	Left	10	Small portion	Good	0.341
182	Right	22	None	Fair	0.158
182	Left	22	Fragment	Fair	0.352
177	Right	26	Few cells	Poor	0.12
177	Left	26	None	Fair	0.341
176		30	None		0.29

The cortex from the adrenal of another cat gave similar results. After the gland had been carefully frozen, the cortex on the side opposite the vein was carefully cut away with a scalpel. The medulla was avoided as before. Extract of this material gave a marked inhibition of kitten's intestine.

We tested for epinephrin in the cortex of adrenals, the medulla of which had been destroyed by cauterization as described above.

Cat 176, whose left adrenal had been cauterized twice, the second time thoroughly, finally died from insufficiency hastened by testing in the treadmill. The second cautery had taken place 10 days before death and had been so thorough that less than 0.044 gram of cortex was alive. Ringer's extract of the crushed cortex produced inhibition of the kitten's intestine in repeated tests.

Cat 178, which had both adrenals cauterized, died 6 days after the removal of the left gland. Cautery of the right adrenal had been so thorough that only a small fragment of the cortex remained. An extract of this caused some inhibition of a piece of rat's intestine.

Although these experiments seemed to indicate that epinephrin is formed by the cortex there is the possibility that the action was not specific because extract of spleen will sometimes produce a similar effect. We therefore abandoned this method as inconclusive.

Production of epinephrin by the cortex as indicated by the exercise test. We next used the sensitized iris (superior cervical ganglion removed several days previously) as a test for epinephrin. It has been shown that the sensitized pupil will dilate during exercise when the adrenals are intact (5). However, when the influence of the central nervous system is removed (by cutting the nerves to the adrenal), this dilatation is usually absent. We attempted to destroy the medulla of both adrenals by cautery. After complete recovery from the operation, the animal was tested in the treadmill and the reaction of the sensitized pupil observed.

Two cats were tested in this way but it was shown later that one of them possessed a piece of medullary tissue. The other cat (175) was tested in the treadmill 10 days after the second cauterization of the remaining adrenal. After 4 minutes during which the cat had travelled 86 meters the sensitized pupil gave a positive test for epinephrin (became larger than control). The dilatation increased very little beyond this, being about the same at 240 meters. This dilatation persisted for approximately 45 seconds following cessation of the exercise. Ten days later that cat died from adrenal insufficiency. No live medulla was found. A small amount of healthy cortex was present. This experiment indicated that epinephrin is produced by the cortex.

This method had one serious objection. If one cauterizes enough of the adrenal to insure complete destruction of the medulla, frequently so much of the cortex is destroyed that the condition borders on insufficiency. Then the animal does not work well in the treadmill. It is on this account more difficult to obtain a dilatation of the sensitized

pupil, although the power of epinephrin production might be present in the cortex. We therefore chose a different method for stimulating the adrenal. This method follows.

Production of epinephrin by the cortex as indicated by the excitement test. Again we used the sensitized iris as the test object. But the ciliary ganglion was removed in order to eliminate nervous influence when employing the stimulus of excitement.

Of all forms of harmless stimuli the excitement produced by shutting off the air from the lungs for 40 seconds will produce the most marked effect in epinephrin secretion (6). Moreover the shutting off of the air rarely ever fails to produce an effect. That it is the excitement rather than asphyxia which produces the effect is indicated by the observation that there is no epinephrin increase when the animal does not become excited. Thus occasionally a cat has not objected to the air being shut off for 40 seconds. There was no change in the completely denervated pupil. The same cat tested at other times has become excited during the shutting off of the air for the same period. Then there was a marked dilatation of the completely denervated pupil. The excitement test was made by holding a rubber glove tightly over the nose and mouth so that it completely shut off the air to the lungs. During the process the legs and head were held firmly by assistants. The transverse diameter of the denervated pupil was measured with a caliper square graduated in 0.01 inch or 0.25 mm.

Our experiments have been conducted according to the following plan. The iris was first sensitized by removal of the superior cervical ganglion. A week or more later, the medulla of the left adrenal was destroyed by electric cautery through the lumbar path without entering the peritoneal cavity. After recovery from the second operation the ciliary ganglion supplying the sensitized iris was removed. Usually within one or two days the response of the completely denervated pupil to the excitement of shutting off air to the lungs for 40 seconds was tested. The right adrenal was next removed through an opening in the mid-line of the abdomen. After recovery from anesthesia the denervated pupil response was tested in a similar manner (air off for 40 seconds). The cat was again anesthetized and the left adrenal removed through the reopened mid-line incision. When the animal had recovered from anesthesia the denervated pupil response was again tested as before, care being taken to have all conditions identical. This test served as a control.

The left adrenal was fixed with formaldehyde and potassium bichromate. Sections 25μ in thickness were cut with a freezing microtome. Approximately every fifth and sixth section were kept for study. After staining with hematoxylin and Sudan III these sections were systematically examined for medullary cells.

We succeeded in completing experiments on fourteen cats. Four of these showed no change in the denervated pupil in response to shutting off air for 40 seconds when only the left cauterized adrenal remained. Before removal of the right adrenal the denervated pupil response to the same test had been marked. In one of these cats, the left adrenal appeared to have been completely destroyed by cautery while in the other three the destruction was nearly complete, only a very small amount of cortex remaining.

In five cats a small piece of medulla had escaped cautery (left adrenal). The epinephrin released as a result of the excitement test was less when the good adrenal (right) was removed as compared with the response before its removal (table 2). After removal of the cauterized adrenal the excitement test produced a much smaller effect or no effect at all on the denervated pupil. In one instance (267) it actually produced constriction of the pupil. The left adrenal had been cauterized from 14 to 24 days previously in this group of cats. The tests following removal of the right and of the left adrenal were made at least 2 to 3 hours after the operation. The only value of these experiments is that they suggest the possibility of epinephrin production by the cortex because after destruction of a large proportion of the medulla, one can still obtain an appreciable output of epinephrin from appropriate stimulation.

Experiments with five cats were conclusive. A careful study of the sections from the cauterized adrenal (left) showed the entire absence of medullary tissue. While the cat possessed only the cauterized adrenal a decided dilatation of the completely denervated pupil could be obtained when air to the lungs was shut off for 40 seconds (table 3). After removal of the cauterized adrenal the same test produced no response in the denervated pupil except in cat 270. In this one animal the response, however, was much less (7.10 to 10.66 mm. before and 7.10 to 7.87) after removal of the cauterized adrenal. The cats were completely out of the influence of ether and the development of excitement as indicated by struggling was carefully observed. Moreover excitement developed just as early and lasted just as long in the last test as in the preceding test. In fact cat 269 struggled more than usual in the last test.

TABLE 2

Effect of removal of adrenal tissue on the response of the completely denervated pupil to the "excitement" test

CAT	CHANGE IN DIAMETER OF THE PUPIL BEFORE REMOVAL OF RIGHT ADRENAL	CHANGE IN DIAMETER OF THE PUPIL AFTER REMOVAL OF RIGHT ADRENAL	CHANGE IN DIAMETER OF THE PUPIL AFTER REMOVAL OF LEFT ADRENAL	CONDITION OF LEFT ADRENAL	
				Medulla present	Condition and amount of cortex
	mm.	mm.	mm.		
261	9.91 to 12.93	9.91 to 11.16	9.39 to 9.66	Small piece	Not more than $\frac{1}{3}$ of normal amount, but healthy
265	2.28 to 4.83	3.55 to 6.60	3.55 to 4.83	Very small piece	Much good cortex
267	3.05 to 12.93	4.32 to 7.87	4.32 to 3.55	Small piece	Much good cortex
271	11.91 to 14.45	12.18 to 13.70	12.18 to 12.43	Small piece	Fair condition
272	7.87 to 12.93	8.12 to 11.91	7.10 to 7.62	About $\frac{1}{3}$ of normal amount	Cortex in excellent condition

TABLE 3

Effect of removal of adrenal tissue on the response of the completely denervated pupil to the "excitement" test. The medulla of the left adrenal was completely destroyed

CAT	CHANGE IN DIAMETER OF THE PUPIL BEFORE REMOVAL OF RIGHT ADRENAL	CHANGE IN DIAMETER OF THE PUPIL AFTER REMOVAL OF RIGHT ADRENAL	CHANGE IN DIAMETER OF THE PUPIL AFTER REMOVAL OF LEFT ADRENAL	CONDITION OF CORTEX IN LEFT ADRENAL
	mm.	mm.	mm.	
268	10.66 to 13.20	10.66 to 11.41	10.66 No change (air off 50 seconds)	Lipoids plentiful
269	10.91 to 11.91	10.16 to 11.16	9.91 No change	Lipoids plentiful
270	7.10 to 12.18	7.10 to 10.66	7.10 to 7.87	Lipoids plentiful
273	9.66 to 12.18	10.16 to 10.91	10.16 No change	Lipoids scarce
279	9.91 to 12.43	9.39 to 10.16	9.66 No change	Lipoids plentiful

The only explanation which can be offered in this last group of animals is that the cortex pours something into the blood stream which causes dilatation of the denervated pupil. We are justified in assuming that it is epinephrin.

DISCUSSION. Our evidence seems to prove that epinephrin is produced in the adrenal cortex. No other substance, so far as we know,

can produce all of the reactions which we have employed; viz., the blue color with the phosphotungstic reagent, inhibition of the intestine and dilatation of the denervated pupil. Absolute proof is to be obtained only by the chemical isolation of epinephrin from adrenals deprived of their medullary tissue.

Epinephrin does not accumulate in the cortex to any extent. In our experiments we have found little more than 0.5 mgm. per gram of tissues as a maximum. It may often be much less than this. It is on this account that it has been difficult to demonstrate the presence of epinephrin in the cortex. A method which tests the epinephrin set free over a short period of time, such as the eye reaction, is undoubtedly more sensitive than one which can use only the epinephrin found in the cortex at any one instant.

Because epinephrin has been found in relatively large amounts in the medulla it has been generally concluded that it is manufactured there. It has, however, been admitted that precursors of epinephrin may be formed in the cortex. Cramer (3), as stated in the introduction, has found what appeared to be epinephrin granules in the cortex. These were more numerous in the regions nearest the medulla suggesting production in the cortex and storage in the medulla.

Further support of the idea that epinephrin is produced in the cortex is obtained in certain conditions which caused an increase in the demand for epinephrin.

First of all let us take muscular exercise. This is accompanied by an increase in the output of epinephrin (5). Excessive exercise leads to vacuolization of the cortex (7) and reduction of the lipoids (8). On the other hand, a gradually increasing demand on this function as undoubtedly occurs with greater muscular development seems to lead to a relative increase in the cortex. At least Elliott and Tuckett (9) have found that during the development of the muscles the cortex increases considerably, while the medulla changes but slightly. Other conditions which increase the demand for epinephrin are also accompanied by marked changes in the cortex. Burns, like other painful stimuli (6), increase the epinephrin output. Thus we have obtained dilatation of the completely denervated pupil in a cat, under the influence of ether, when a small area of the skin has been exposed to water at 100°C., for one second or less. On the next day, a few hours after the removal of both adrenals, similar stimulation produced no effect on the denervated pupil. After severe burns the cortex becomes hemorrhagic and shows exhaustion of lipoid in certain areas (10).

In brief, then, any condition which calls forth a marked increase in the epinephrin output for a considerable period of time seems to affect the cortex. This might be due to an excessive activity of the cortex in producing epinephrin.

SUMMARY

The Folin, Cannon and Denis colorimetric test for epinephrin has been obtained from the cortex prepared either by shaving it away from the medulla while frozen or by complete cauterization of the medulla some time previously. According to this method epinephrin occurs to the extent of 0.5 mgm. per gram or less in the cortex.

Inhibition of contracting intestine has been obtained from extracts of cortex prepared by the methods just described.

Dilatation from exercise has been obtained in the sensitized pupil after one adrenal has been removed and the medulla of the other destroyed by cauterization.

Dilatation of the completely denervated pupil in response to the excitement caused by shutting off air from the lungs for 40 seconds has been obtained in animals possessing but a single adrenal and that with medulla completely destroyed. After the cauterized adrenal had been removed a similar test produced either no reaction or only a slight one.

Epinephrin, therefore, appears to be produced in the adrenal cortex.

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Contents of Volume III, 1923

JANUARY

- A. J. CARLSON: The Gastric Secretion in Health and Disease
J. A. GUNN: Cellular Immunity
E. A. PARK: The Etiology of Rickets
PEYTON ROUS: Destruction of Red Blood Corpuscles in Health and Disease

APRIL

- W. MCK. MARRIOTT: Anhydremia
YANDELL HENDERSON: Volume Changes of the Heart
W. T. LONGCOPE: Hyper-sensitization in Man and its Relation to Disease
F. H. PIKE: Functions of the Vestibular Apparatus

JULY

- H. H. DALE: Chemotherapy
D. W. WILSON: Neutrality Regulations in the Body
F. H. A. MARSHALL: Internal Secretions of the Reproductive Organs
P. A. SHAFFER: Intermediary Metabolism of the Carbohydrates
A. I. KENDALL: Bacterial Metabolism

OCTOBER

- WM. C. ROSE: Purin Metabolism
T. H. MORGAN: The Mechanism of Heredity
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